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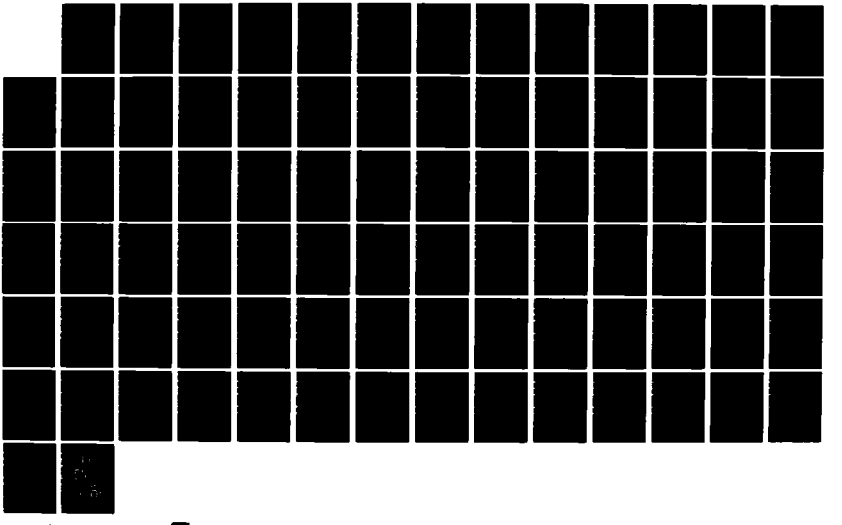

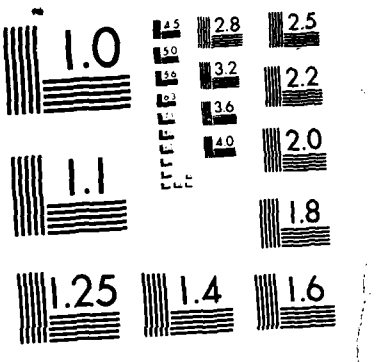

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COMPARISON OF HEAVY METAL UPTAKE BY *EISENIA FOETIDA* WITH THAT OF OTHER COMMON EARTHWORMS

Final technical report

by

Elizabeth A. Stafford and Clive A. Edwards

Entomology Department,

Rothamsted Experimental Station,

Harpenden, Herts. AL5 2JQ, U.K.

December 1985

Contract number DAJA 45-84-C-0027

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Earthworms have been used in the field to indicate levels of soil pollution and in the laboratory for the ecotoxicological testing of industrial chemicals. An earthworm bioassay procedure developed at the Waterways Experiment Station (Vicksburg,Mississippi) was modified and evaluated as a method of providing information on heavy metal bioavailability in contaminated soils and sediments from Europe. Eight soils/sediments containing elevated levels of at least one of the elements Zn, Cu, Cd and

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Pb were selected as well as a control and a reference soil. Six species of earthworm, including the WES bioassay earthworm E.foetida, and five field species were grown in the soils/sediments for periods of 15, 28 or 56 days. Concentrations of the elements Zn, Cu, Cd, Ni, Cr and Pb present in the earthworm samples (corrected for the presence of soil-derived metals within the earthworm gut) were compared between earthworm species from the same soil and for each earthworm species from a range of metal contaminated soils/sediments. A close linear relationship between metal uptake by E.foetida and the field species of earthworm emerged and good correlation between total (HNO_3/$HClO_4$) soil Pb and Cd levels and earthworm tissue concentrations and between DTPA extractable soil Cu and Cd levels and earthworm tissue concentrations was observed.

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ABSTRACT

Earthworms have been used in the field to indicate levels of soil pollution and in the laboratory for the ecotoxicological testing of industrial chemicals. An earthworm bioassay procedure developed at the Waterways Experiment Station (Vicksburg, Mississippi) was modified and evaluated as a method of providing information on heavy metal bioavailability in contaminated soils and sediments from Europe. Eight soils/sediments containing elevated levels of at least one of the elements Zn, Cu, Cd and Pb were selected as well as a control and a reference soil. Six species of earthworm, including the WES bioassay earthworm E. foetida, and five field species were grown in the soils/sediments for periods of 15, 28 or 56 days. Concentrations of the elements Zn, Cu, Cd, Ni, Cr and Pb, present in the earthworm samples (corrected for the presence of soil-derived metals within the earthworm gut) were compared between earthworm species from the same soil and for each earthworm species from a range of metal contaminated soils/sediments. A close linear relationship between metal uptake by E. foetida and the field species of earthworm emerged and good correlation between total ($HNO_3/HClO_4$) soil Pb and Cd levels and earthworm tissue concentrations and between DTPA extractable soil Cu and Cd levels and earthworm tissue concentrations was observed.

1. INTRODUCTION

Dredging operations, undertaken by the Army Corps of Engineers in order to maintain navigable channels through the harbours and waterways of the United States, generate over 2,000,000,000 m^3 of dredged material to be disposed of annually (U.S. Army Engineer Water Resources Support Center, 1979). These dredged sediments are often contaminated as a result of industrial discharges, agricultural run-off, sewage effluents and mining operations. Depending on the source of the sediment and the availability of suitable sites, the dredged material may be disposed of at sea or by the creation of wetland or upland disposal sites. The choice of site for disposal and the management strategy adopted at the site once established will depend upon the level and mobility of contaminants present in the dredged material. An assessment of contaminant mobility and bioavailability can be made by monitoring the uptake into plant and animal tissues.

The Waterways Experiment Station (Vicksburg, Mississippi) has developed plant and animal bioassay procedures using surrogate species of plant and animal to indicate the bioavailability of contaminants present in the dredged material. One of the animal bioassay procedures uses the earthworm Eisenia foetida grown in dredged sediments to be disposed of in upland sites.

Earthworms possess many characteristics which make them ideally suited for use as bioassay and bioindicator organisms of contaminant bioavailability (Ma, 1982). This has led to their use in numerous studies to provide an assessment of biologically available levels of pollutants within various substrates (Czarnowska and Jopkiewicz, 1978; Atlanvinyte et al., 1980; Carter et al., 1980; Marquenie and Simmers, 1948; Pietz et al., 1984) and to their being recommended for use as a key indicator organism in the EEC/OECD procedure for the ecotoxicological testing of industrial chemicals (EEC Directive 79/81, 1984).

Earthworms are known to exploit a wide range of ecological niches within the soil profile (Edwards and Lofty, 1977). For example : the bioassay worm E. foetida is common in substrates rich in organic matter with little or no mineral soil while many of the field species burrow within the mineral soil profile ingesting some proportion of decaying organic matter. These differences in behaviour and feeding habits between earthworm species are known to influence the anatomy of the gut (Semenova, 1966) and may also influence the uptake of metals from soil which is ingested (Ireland, 1979; Ireland and Richards, 1981). Therefore the use of a surrogate species (*E. foetida*) in studies of bioavailability needs to be validated by making inter-specific comparisons of metal uptake by earthworms with a range of behavioural and feeding habits.

The research objectives of the present study may be summarized as follows:-

(1) To assess the suitability of using earthworms, in particular the species E. foetida, to indicate bioavailability of the heavy metals Zn, Cu, Ni, Cd, Cr and Pb in soils and sediments.

(2) To assess the suitability of using E. foetida as a surrogate species to indicate the bioavailability of Zn, Cu, Ni, Cd, Cr and Pb present in soils and sediments, to other earthworm species.

2. MATERIALS AND METHODS

2.1 SOILS AND SEDIMENTS

Soils and sediments used in the present study were selected by research workers based in Wales and in the Netherlands where plant bioassays were conducted to monitor the uptake of heavy metals by agronomic plants and the WES reference plant: Cypereus esculentus. All plants were grown in soils and sediments with a wide range of contamination by heavy metals and containing elevated levels of at least one of the elements Zn, Cu, Cd and Pb. A full description of the sources and sites from which the ten soils used in the present experiment were collected is given elsewhere (Davies and Houghton, 1983; Van Driel et al., 1983) and only a brief summary is provided in Table 2.1.

Table 2.1 Origin of experimental soils and sediments

SITE	ABBREVIATION	SOURCE	DETAILS
FRONGOCH	F	Aberystwyth Dyfed, Wales	Control soil-containing low levels of heavy metals. Local Aberystwyth soils are Entisols or Inceptisols developed in Silurian grits and shales or Pieistocene deposits with a shale matrix.
YSTWYTH	Y	Ystwyth Valley Dyfed, Wales	Area of Pb and Zn mining in the 19th Century. Spoil heaps and effluent from inefficient ore-processing has resulted in widespread contamination of valley soils.
HALKYN MOUNTAIN	HMt	Halkyn Mountain North Wales	Carboniferous limestone mineralized by ores of lead (galena PbS) and zinc (sphalerite ZnS). Inefficient extraction processes have lead to widespread contamination of soils by Pb and Zn.
SHIPHAM	S	Shipham, Somerset, England	The main ore mined in the area is Smithsonite ($ZnCO_3$) and the mineral is especially rich in cadmium.
PARYS MOUNTAIN	PMt	Parys Mt Angelsey, U.K.	In the 18th Century this area was the largest supplier to the world copper market. Mining activities ceased in 1883 but local soils have elevated levels of Cu and other heavy metals

Table 2.1 (continued)

SITE	ABBREVIATION	SOURCE	DETAILS
BROEKPOLDER	BP	Nr Vlaardingen, Netherlands	A disposal site for dredged materials from Rotterdam harbour. Last disposal of dredge spoils 1975
OOSTABTSPOLDER	AP	Nr Rotterdam, Netherlands	A disposal site for dredged materials from Rotterdam harbour. Last disposal of dredge spoils 1974.
SPIERINGPOLDER	SP	Reclaimed from freshwater tidal area of Rivers Rhine & Meuse, Netherlands	Polder diked in 1953 contains young river clay sedimented in situ.
NECKAR SLUDGE	NS	Nr Lauffen, River Neckar, Federal Republic of Germany	R. Neckar is a tributary to the river Rhine. The dredged sediment was dumped on land in 1979.
WES REFERENCE	WES	Vicksburg, Mississippi, U.S.A.	A poor, sandy loam soil derived from lake sediment.

In preparation for experimental use all soils were air dried and passed through a 2 mm mesh stainless steel sieve. The soil fraction > 2mm was discarded.

2.2 EARTHWORMS

The five species of earthworm selected for comparison with the bioassay earthworm *E. foetida* are listed below together with a note on their natural habitat and feeding preferences (Arthur, 1965; Gerard, 1964; Bouche, 1972; Piearce, 1972):

Lumbricus terrestris (Linnaeus, 1758) - Soil-inhabiting, deep burrowing species, moving to the surface to feed on litter. Colonizing gardens, arable and pastureland, forests and river banks. Abundant in clay soils.

Lumbricus rubellus (Hoffmeister, 1845) - Colonizing substrates rich in organic matter and usually wet. Abundant in parks, gardens and pastures.

Allolobophora longa (Ude, 1885) - Soil inhabiting, deep burrowing species, ingesting predominantly mineral soil. Colonizing cultivated soil, gardens, pastures and woodland. Abundant in chalky soils.

Allolobophora caliginosa (Savigny, 1826) - Predominantly inhabiting the mineral soil horizon. Abundant in cultivated soils, parks, gardens and pastures. Mainly in chalky soils.

Allolobophora chlorotica (Savigny, 1826) - Surface dwelling species feeding on litter and ingesting very little mineral soil. Abundant in gardens, pasture and arable land.

Eisenia foetida (Savigny, 1826) - Generally found in soil rich in organic matter and in manure and compost heaps.

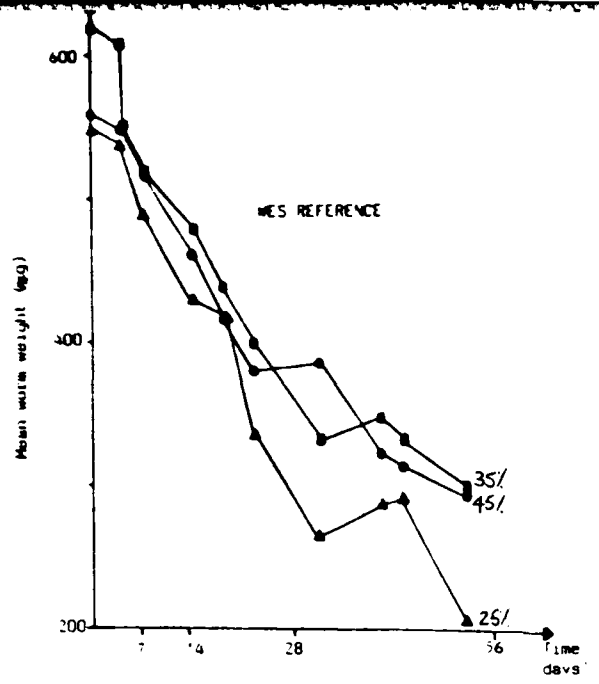
Since intra-site comparisons have shown that levels of metals can differ significantly within species between adult and sub-adult stages (Ma, 1983) only adult, clitellate earthworms were used in the present experiment. Some bioaccumulation of heavy metals is likely to have occurred during the life span of these earthworms and high base line levels of metal elements have been reported in adult earthworms taken from unpolluted soils (Andersen, 1979; Ash and Lee, 1980; Ma, 1982). *L. terrestris*, *A. longa*, *A. caliginosa* and *A. chlorotica* for this experiment were collected from Rothamsted Park, a soil of similar heavy metal composition to the control soil: Frongoch (Table 2.2). These field species of earthworm were obtained by application of 0.5% formaldehyde solution to the soil (Raw, 1959). Emerging earthworms were immediately rinsed free of the chemical in tap water and held for up to one week in an artificial soil mix* until sufficient populations of each species were available.

Table 2.2 Concentrations of heavy metals measured in substrates from which experimental earthworms were collected compared with the control soil: Frongoch ($\mu g\ g^{-1}$, dry weight)

	Earthworm substrate		
Element	Rothamsted Park	Cattle Waste	Frongoch
Zn	120.47	171.43	115.98
Cu	26.90	38.10	22.89
Ni	30.85	3.95	26.39
Cd	<0.13	0.17	0.91
Cr	45.14	7.06	41.85
Pb	52.58	9.04	75.87

E. foetida were collected from cattle waste which had been analysed to ensure low levels of metal contaminants (Table 2.2) and L. rubellus were collected from horse manure at a local stables. Insufficient populations of L. rubellus were available for satisfactory inclusion in the experiment. Earthworms from each source were oven dried at 85°C and analysed for heavy metal content.

*Artificial Soil = 10% peat; 20% kaolinite clay; 69% quartz sand; ~1% $CaCO_3$ (% dry weight) (EEC Directive 79/831) Analysis ($\mu g\ g^{-1}$, dry weight) = Zn = 9 $\mu g\ g^{-1}$; Cu = 3 $\mu g\ g^{-1}$; Cd = < 0.13 $\mu g\ g^{-1}$; Pb = 13 $\mu g\ g^{-1}$.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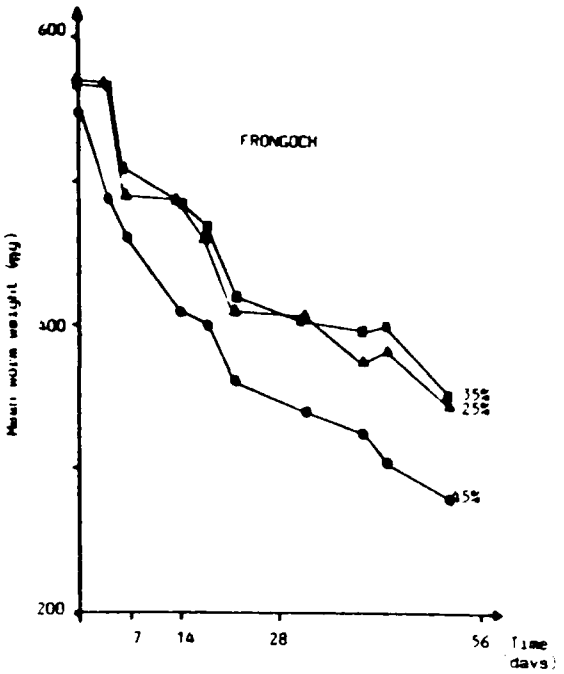
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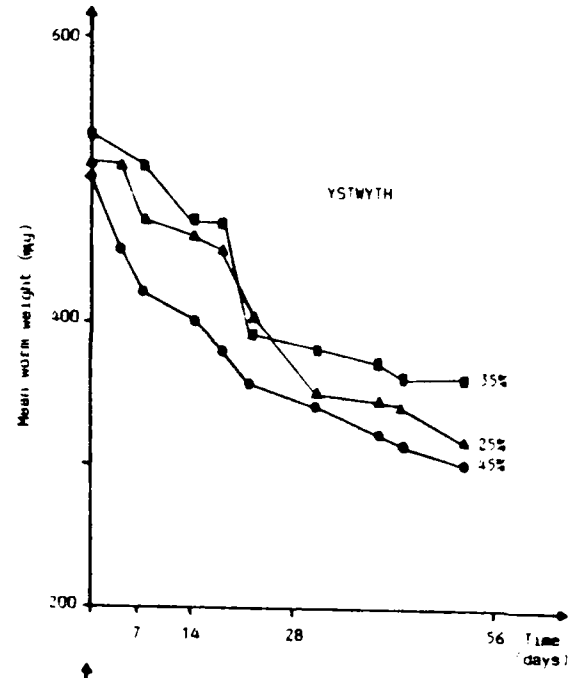
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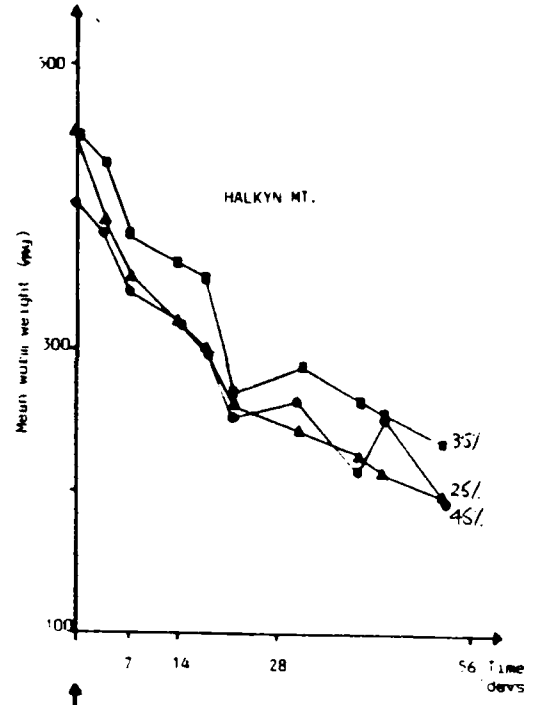
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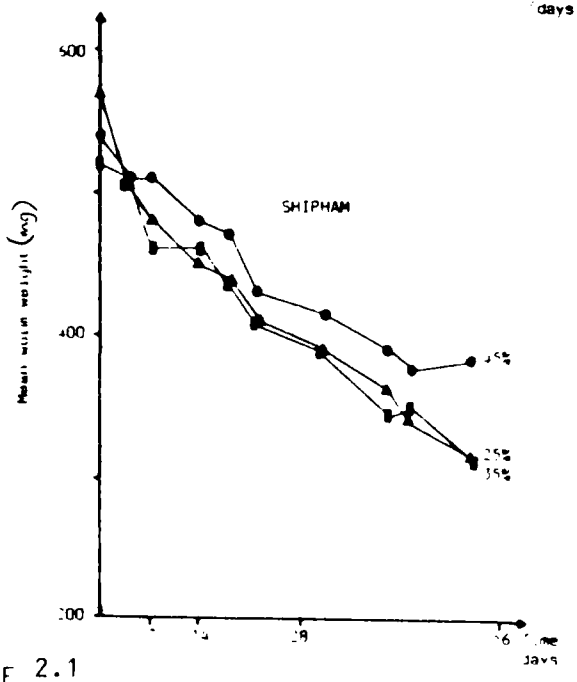
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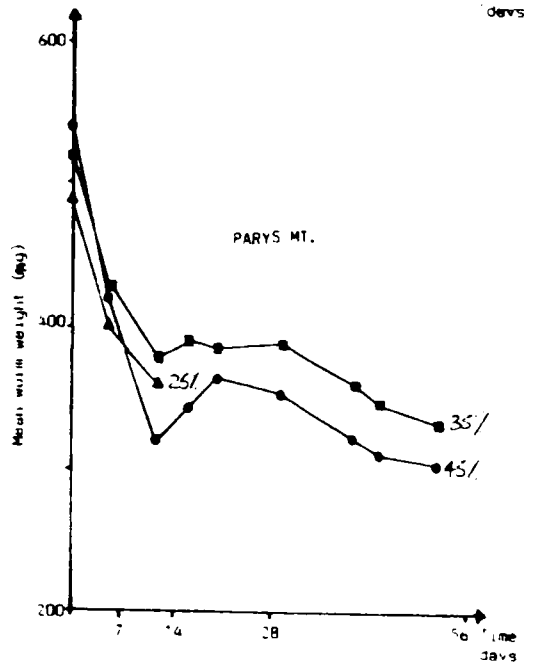
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FIGURE 2.1

2.3 EXPERIMENTAL PROCEDURE

The earthworm bioassay procedure was developed at WES using *E. foetida* and some modifications were considered necessary for its application to the field species of earthworm and to ensure valid comparisons between species. Some preliminary investigations were therefore carried out before conducting a large scale experiment:-

2.3.1 Screening of substrates for toxicity to earthworms: Five earthworms of each species were placed onto 100 g sub-samples of each soil and sediment in triplicate (rewetted to field capacity). The initial burrowing activity of the worms was observed and subsequent mortality and physical condition checked after 4, 7 and 14 days. Only the Parys Mt. soil was toxic to earthworms with 100% mortality recorded after 4 days. This may have been due to the low pH of this soil (3.9) or due to Cu toxicity at this low pH. Zero earthworm mortality in this soil was achieved by addition of 0.01% $CaCO_3$ to increase the soil pH.

2.3.2 Physical conditions for the bioassay

(a) Optimum moisture content:- Five earthworms (*E. foetida*) were added to 100 g sub-samples of each of the soils rewetted to 25%, 35%, and 45% moisture and each replicated three times. The rate of weight loss of the earthworms was used as an indication of the stress to which the worms were subject under different moisture conditions (Figure 2.1). In each case, with the exception of the Shipham soil and the WES reference soil, minimum weight loss was recorded in worms held at 35% moisture. However, at 35% moisture, standing water was present in some of the containers. Variation in water holding capacity between soils ruled out the use of one moisture content for all soils and since there was little difference in the rate of earthworm weight loss for moisture contents of 25-45% all soils were rewetted to approximately 30% moisture content for use in the bioassay.

(b) Food supply for earthworms: Excessive weight loss by earthworms during the bioassay was considered unsatisfactory and the provision of a food supply for the earthworms suggested. For example, corn meal has been used in toxicity tests using *L. terrestris* (Karnak and Hamelink, 1982). Corn meal, pure cellulose and a commercially available dried grass were mixed with the Frongoch and Ystwyth soils at levels of 0% (control); 1%; 2.5% and 5% (by weight). Four *E. foetida* were added to 100 g of each soil + feed combination in triplicate and their percentage weight loss measured after 56 days (Table 2.3). Addition of corn meal or cellulose to the soil resulted in an increase in weight of earthworms after 56 days compared with soil to which no feed was added. Addition of feed materials to the soil is likely to affect the results of the earthworm bioassay since selective feeding by earthworms may reduce the quantity of soil ingested during the experimental period and binding properties of the feed may result in heavy metals being biologically unavailable for uptake. It was considered likely that the addition of feed material to the soil would affect results of a bioassay at least as much as the weight loss by earthworms over the experimental period and subsequently no food was added to soils used in the bioassay.

Table 2.3: Percentage weight change of *E. foetida* grown for 56 days in soil to which a food supply had been added

FEED TYPE/ SOIL		LEVEL OF FEED ADDED 0%	1%	2.5%	5%
NO FOOD	FRONGOCH	-28.9			
	YSTWYTH	-28.9			
GRASS	FRONGOCH		-12.9	+14.3	+16.1
	YSTWYTH		-16.7	+3.3	-28.1
CORN MEAL	FRONGOCH		0.0	+22.0	+51.6
	YSTWYTH		+13.8	+32.3	mortality
CELLULOSE	FRONGOCH		+16.7	+22.0	+38.7
	YSTWYTH		+13.8	+51.6	+43.3

(- ve = weight loss; + ve = weight gain)

(c) Density of earthworms : soil: Earthworm : soil density will influence the food supply available to worms and high stocking densities for example 4-5 worms/100 g soil/56 days, as used above, may result in weight loss by earthworms. Rates of soil consumption by earthworms have been estimated in the laboratory at 240 mg soil/*L. terrestris* (4 g, live wt.)/day (Curry and Bolger, 1984) and 16 mg soil/*E. foetida* (300 mg, live wt)/ day (calculated from Hartenstein *et al*, 1981). Previous studies measuring uptake of contaminants by earthworms have used the following stocking densities:-

Reference	Earthworm species (& live wt)	Stocking density
Gissel-Nielsen & Gissel-Nielsen (1975)	*L. terrestris*	8 kg soil/8 worms/85 days 2 l soil/8 worms/85 days 201 soil/10 worms/3 months
Mori & Kurihara (1979)	*E. foetida*	500g compost/10 worms/60 days
Atlavinyte *et al* (1980)	Mixed species	3 kg soil/10-20 worms/ 20-60 days
Fleckenstein & Graff (1982)	*E. foetida*	15 kg waste/370 worms/ up to 216 days
Ma (1982)	*L. rubellus* (~700 mg)	5 l soil/5 worms/6-12 weeks
Maleki *et al* (1982)	*E. foetida*	50 g manure + soil/2 worms 8 weeks (20 g manure replenished after 4 and 6 weeks)
EEC Test (1984)	*E. foetida*	500g substrate/10 worms/14 days
Marquenie and Simmers (1984)	*E. foetida*	7 l soil/150 worms (20±0.5g)/ 49 days
Neuhauser *et al* (1985)	*E. foetida* (300-500 mg)	400g soil/10 worms/14 days

Based on the EEC test stipulations and backed up by the results of other research, a stocking density of 1 g worm/40 g soil (live weight)/week was chosen for subsequent bioassays. A 28 day test was conducted to measure the rate of weight change of *E. foetida* held in Ystwyth soil at this stocking density. For comparison, and to assess the effect of adding feed to the soil at lower stocking densities, 1% (w/w) pure cellulose was mixed with soil for one set of replicates. The results of this 28 day test are shown below (Table 2.4). It appeared that by decreasing the population density, sufficient nutrient was available in the Ystwyth soil to maintain earthworm biomass. Lower stocking densities also decrease the likelihood of earthworms ingesting soil more than once, which may affect metal uptake.

Table 2.4 Weight change of E. foetida grown in Ystwyth soil (with and without the addition of food) at a stocking density of 40 g soil/g live weight worm/week.

	TIME (Days)				
	0	7	14	21	28
NO FOOD	0.319 (0.020)	0.347 (0.037)	0.334 (0.041)	0.330 (0.049)	0.324 (0.039)
WITH FOOD	0.321 (0.028)	0.371 (0.034)	0.379 (0.029)	0.373 (0.043)	0.371 (0.047)

Standard deviation of the mean given in parenthesis

(d) Temperature: The optimum temperature for laboratory culture varies from species to species : 4-6°C for *L. terrestris* (Tomlin 1977) to 18-25°C for *E. foetida* (Hartenstein, 1983). However, field species of earthworm have been successfully cultured in the laboratory at 15°C (Lofs-Holmin, 1982) and used for long term toxicity tests (96 days) at 15°C (Lofs-Holmin, 1980). A constant temperature of 15°C was considered acceptable for all species used in the present experiment and all bioassays were conducted at this temperature.

2.3.3 Experimental Design

AIM - Measurement of heavy metal uptake by six species of earthworm after three experimental periods in ten soils/sediments.

5-8 g of earthworms (live weight) was considered adequate for the chemical analysis at the end of the experiment. At the selected stocking density, this weight of earthworms would require approximately 600 g; 1,200 g and 2,400 g of soil for experimental periods of 15, 28 and 56 days respectively. For each combination of earthworm species and soil, separate containers were filled with 600 g soil (Day 15 sample); 1,200 g soil (Day 28 sample) and 2,400 g soil (Day 56 sample) each replicated three times. The experimental design is represented in Figure 2.2. Insufficient materials were available to conduct bioassays using all the combinations of soil and earthworm species.

All soils were rewetted and 5-8 g and earthworms added. Earthworms which had not burrowed into the soil within five minutes were replaced. The number of earthworms required for each container of soil was estimated as follows:-

Earthworm species	Approximate weight/worm	Number of worms/container	Total weight of worms/container (g)
E. foetida	300 - 500 mg	15	4.5 - 7.5
L. terrestris	2 - 4 g	3	6 - 12
A. longa	1 - 3 g	6	6 - 18
A. caliginosa	300 - 500 mg	15	4.5 - 7.5
A. chlorotica	200 - 300 mg	20	4 - 6
L. rubellus	300 - 500 mg	$\propto$ availability	

At the end of each experimental period earthworms were hand sorted from the soil, counted, weighed and oven dried at 85°C. Dried samples were ground to a fine powder and used for the determination of acid insoluble residue and heavy metal content.

2.3.4 Gut depuration: Earthworms sampled after each bioassay were immediately killed and used for heavy metal analysis without allowing time for gut depuration. Bioavailability of heavy metals in the soils and sediments was assessed in terms of uptake into the earthworm tissue. It was therefore essential to distinguish between metal concentration in the sample, present in the earthworm tissue (bioavailable) and metal concentration in the sample due to soil within the earthworm gut (not necessarily bioavailable).

A method has been developed which utilises acid insoluble residue as an inert marker to enable the quantity of soil present in any earthworm sample to be calculated. A correction factor can then be applied which eliminates the heavy metal concentration resulting from soil within the earthworm gut, leaving only the concentration of heavy metals incorporated into the earthworm tissue.

Details of this method have been submitted for publication and to avoid repetition a pre-print of this paper is included in the appendix of this report.

2.4 Chemical Methods

Soil and sediment analysis

Physical and chemical analysis of the samples was carried out using oven dried samples of < 2 mm particle size. Due care was taken during all sample preparation to avoid contamination by heavy metals.

DTPA - extractable metals were measured by a procedure based on the method of Lindsay and Norvell (1978) and modified by Lee *et al* (1978): 20 ml of a 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M triethanolamine solution, buffered at pH 7.3, was added to 10 g samples of soil in plastic centrifuge tubes. Samples were placed in an automatic shaking machine for 2 hours

exactly and filtered through ashless filter paper (Whatman No. 42). Concentrations of Ca, Fe, Zn, Cu, Ni, Cd, Cr and Pb were determined in the filtrate by inductively coupled plasma (ICP) emission spectrometry (ARL 34000 instrument) with background correction. Standard solutions of these elements were prepared using the same extractant solution and reagent blanks were also run.

Total metal concentrations in the oven dried soil and sediment samples were determined by a wet ash procedure: ~ 0.5 g samples were digested in 'Analar' grade concentrated HNO_3 (8 ml) for 5 hours at 125°C. After cooling, 'Analar' grade 70% $HClO_4$ (1 ml) was added before re-heating to 200°C, taking samples almost to dryness. Samples were re-extracted in hot 5% HCl and concentrations of Ca, Fe, Zn, Cu, Ni, Cd, Cr and Pb determined by ICP, with background correction. Standard solutions of elements measured were made up in 5% HCl. Reagent blanks were also run.

Acid insoluble residue (AIR) - approximately 0.5 g oven dried soil samples were ashed at 450°C overnight, the ash digested in 5 ml 6N HCl at 100°C for 1 hour and taken to dryness. Samples were re-extracted in hot 5% HCl, filtered and washed free of acid through Whatman No. 42 paper. Residue and filter paper were re-ignited at 600°C and AIR determined gravimetrically as a percentage of oven dried sample. Acid and filter paper blanks were also run.

Earthworm analysis

Acid insoluble residue and heavy metal concentrations of finely ground, oven dried earthworm samples were determined using the methods described above.

2.5 Statistical Methods

Linear regression analyses of the data and correlation coefficients were calculated to:
(i) determine the significance of the relationship between soil metal levels (total and DTPA-extractable) and metal concentrations in earthworm tissue.
(ii) compare uptake of metals by E. foetida and the field species of earthworms.

Figure 2.2 Diagramatic representation of the experimental design

SOIL	MAJOR CONTAMINANTS	EARTHWORM SPECIES					
		E. foetida	*L. terrestris*	*A. longa*	*A. caliginosa*	*A. chlorotica*	*L. rubellus*
FRONGOCH	CONTROL	✓ *	✓ *	✓ *	✓ *	✓ *	✓ +
YSTWYTH	Pb, Zn	✓ *	✓ *	✓ *		✓ *	
HALKYN MT	Pb; Zn; (Cu)	✓ *	✓ *	✓ *			
SHIPHAM	Pb; Zn; Cd	✓ *	✓ *	✓ *	✓ *		
PARYS MT	Cu; Pb	✓ *	✓ *	✓ *			
BROEKPOLDER	Pb; Zn; Cu; Cd	✓ *	✓ *	✓ +			✓ +
COSTABTSPOLDER	Zn	✓ *	✓ *	✓ +	✓ +		
SPIERINGPOLDER	Pb; Zn	✓ *	✓ *	✓ +	✓ *		
NECKAR SLUDGE	Zn; Cu; Cd	✓ *	✓ +	✓ *		✓ *	
WES REFERENCE	REFERENCE	✓ *					

/ * Bioassay conducted and earthworms sampled after 15, 28 and 56 days

/+ Bioassay conducted and earthworms sampled after 28 days only

3. RESULTS

3.1 Soil and Sediment Analysis

Physical and chemical characteristics of the experimental soils and sediments are given in Table 3.1. All sediments originating from the Netherlands and Germany were collected from within the same river basin (of the rivers Rhine and Meuse) and had similar physical and chemical properties. By comparison more differences were observed in the physical and chemical characteristics of soils collected in the U.K. (Table 3.1).

A wide range of metal concentrations were present in the soils and sediments from different sources. The total ($HNO_3/HClO_4$) and extractable (DTPA) concentrations of selected metal elements are given in Tables 3.2 and 3.3 respectively. The ratio of DTPA extractable metal: $HNO_3/HClO_4$ extractable metal (Table 3.4) provides some indication of the relative availability of metals in the soil. Very low ratios were recorded for Cr (0.001 or less) and ratios increased in the following order: Cr < Ni < Zn < Pb < Cu < Cd. Consistently low ratios for all elements were evident in the Shipham and Parys Mt soils.

3.2 Earthworm Survival

The percent recovery of earthworms during the experiment was high (Table 3.5) with an overall mean of 92.3%. Loss of earthworms due to mortality or migration occurred equally between species and sampling times. In only four earthworm/soil combinations was an entire replicate lost. A single earthworm mortality can result in the mortality of the entire population and this may explain these losses.

Numerically, no higher percentage recovery was observed on the Day 15 sampling time compared with Day 28 or Day 56. However, recovery of earthworm biomass (expressed as weight per worm) declined noticeably with increasing experimental period (Table 3.5). This decline was highest in earthworms from the Shipham soil and the Neckar Sludge and lowest in the earthworms from the Frongoch soil. Weight loss over the experimental period may be a reflection of the quantity of soil within the worm gut since lower ash and acid insoluble residue contents were also measured in earthworm samples as the experimental period increased. By comparison, biomass measurements for *E. foetida* from the WES reference soil lay mid-range among weight measurements of earthworms from the experimental soils (Table 3.5).

3.3 Heavy Metal Concentrations in Earthworms

Earthworms were killed and analysed for heavy metal content immediately after removal from the soil, without allowing time for gut depuration. Concentrations of heavy metals measured in these samples (earthworms including soil within the gut) are given in the Appendix: Table 1a-f. These results are of interest since they represent the contaminant load passed on to any predator feeding on these earthworms.

However, for more accurate assessment of the bioavailability of these elements it is essential to eliminate the contribution of soil-derived metals. Acid insoluble residue content of soils and samples was used to apply a correction factor to metal concentrations measured in earthworm samples (method given in the appendix of this report,), and the concentration of each element present only in the earthworm tissue is given in Table 3.6, a-f. As pointed out in the description of the method a combination of very high metal concentrations combined with relatively low and insoluble residue content of the soil, as is the case for the Shipham

soil, limits the accuracy of the calculated concentration of metal in the earthworm tissue. This may explain the negative results and due care was therefore taken in interpretation.

3.4 Relationship between Soil and Earthworm Metal Concentrations

The significance of this relationship was assessed by calculating the linear regression of metal concentrations in the earthworm tissue against soil total and DTPA extractable metal levels. The percent variance accounted for by each linear regression of earthworm against soil metal concentration is given in Table 3.7. The extremely high concentrations of total Zn, Pb and Cd measured in the Shipham soil (Table 3.2) resulted in skew data for these elements. All data was therefore converted to natural logarithm before calculating linear regressions for the elements Zn, Pb and Cd.

The linear relationship between earthworm metal concentration and soil metal concentration (total and DTPA extractable) at the Day 28 sampling time is plotted in Figures 3.1 - 3.4 for the elements Zn, Cu, Cd and Pb.

Zinc : Generally, Zn concentrations in the soil (both total and DTPA extractable) were poorly reflected by the earthworm tissue concentrations (Table 3.7; Figure 3.1). Stronger linear relationships were observed between total soil Zn and earthworm tissue concentrations compared with DTPA extractable soil Zn and earthworm tissue concentrations. Of the earthworm species studied, the highest percent variance accounted for by the linear relationship between soil and earthworm Zn concentrations was observed for *E. foetida* (Table 3.7). The slope of the line of best fit indicated that Zn concentrations in earthworm tissue increased slowly with increasing soil Zn levels; with *E. foetida* showing the strongest response (Figure 3.1).

Cadmium: Concentrations of Cd measured in each of the earthworm species were correlated with soil Cd concentrations (total and DTPA extractable (Table 3.7). A closer correlation was noted between earthworm Cd concentrations and total Cd concentration in the soil compared with DTPA extractable Cd in the soil. Tissue Cd concentrations for all earthworm species reflected soil Cd concentrations fairly well, and the slope of the line of best fit was greatest for *E. foetida* (Figure 3.3).

Lead: The results of linear regression analysis between earthworm and soil Pb concentrations showed a similar pattern to that observed for Zn and Cd: closer correlation between earthworm Pb concentrations and soil total Pb levels compared with soil DTPA extractable Pb concentrations. A greater difference in the percent variance accounted for by each linear relationship was present between total and DTPA extractable Pb compared with similar results for Zn and Cd (Table 3.7). Increasing soil Pb concentrations (particularly total Pb) were clearly reflected in the increasing levels of Pb measured in earthworm tissue (Figure 3.4).

Copper: In contrast to the results obtained for Zn, Cd and Pb; Cu concentrations in earthworm tissue were more closely correlated with DTPA extractable Cu levels in the soil compared with total Cu in the soil (Table 3.7). Higher Cu concentrations were recorded in earthworm tissue as the level of Cu in the soil increased (Figure 3.2). A very close linear relationship was recorded between DTPA extractable soil Cu and Cu concentrations in *E. foetida* after 15 and 28 day experimental periods (Table 3.7).

Chromium: Linear regression analyses showed poor correlation between soil Cr levels and earthworm Cr concentrations (Table 3.7). Furthermore

the slope of those lines described by calculation indicated little increase in the earthworm Cr concentrations to correspond with increasing soil Cr levels.

Nickel: Very poor correlations were observed between earthworm and soil Ni concentrations (Table 3.7). For half the comparisons the residual variance exceeded the variance of the Y variate and in only two cases did the percent variance account for over 70% of the relationship.

Table 3.1 Physical and chemical characteristics of the ten experimental soils and sediments

SOIL/SEDIMENT (<2mm)	Particle size (%)*1. 500μm--2mm	200μm--500μm	100μm--200μm	50μm--100μm	16μm--50μm	<2μm--16μm	<2μm	$CaCO_3$ equivalent (%)*2	Organic carbon (%C)*2	pH (1 : 2.5) in water	pH (1:2.5) In 0.01M $CaCl_2$	C.E.C (me/100g)
Frongoch	9	5	2	4	18	38	24	-	4.3	6.0	5.5	20.8
Ystwyth	6	30	<1	<1	23	32	9	-	2.0	6.2	5.4	7.3
Halkyn Mt	4	2	1	4	25	33	31	4.7	2.5	8.1	7.3	18.4
Shipham	26	24	14	8	16	11	1	32	0.7	7.6	7.2	7.7
Parys Mt	19	12	5	6	25	20	13	-	3.6	3.9	3.7	23.5
Broekpolder	<1	2	<1	9	22	35	32	13	5.7	7.4	7.3	29.2
Oostabts-polder	<1	6	<1	<1	23	37	34	14	4.3	7.5	7.4	22.8
Spiering-polder	1	7	11	5	18	29	29	12	3.2	7.8	7.6	25.0
Neckar Sludge	1	3	5	6	26	29	30	16	3.6	7.4	7.2	25.9
WES reference	<1	<1	<1	<1	63	28	9	-	0.6	5.9	5.0	9.5

*1. Particle size separates as a percentage of oven-dry peroxidised soil <2mm
*2. Other percentages based onoven-dry soil <2mm

Table 3.2 Total ($HNO_3/HClO_4$) metal concentrations in the ten experimental soils and sediments ($\mu g\ g^{-1}$, oven dry weight)

SOIL/SEDIMENT	Ca	Fe	ZN	Cu	Ni	Cd	Cr	Pb
Frongoch	2,101.1	40,942.7	115.98	22.89	26.39	0.91	41.85	75.87
Ystwyth	1,267.9	53,083.2	790.23	34.71	39.94	3.02	40.01	1,442.13
Halkyn Mt	6,794.0	20,115.2	1,382.85	119.50	58.47	3.40	39.63	944.91
Shipham	12,972.7	164,530.4	142,673.65	207.07	25.47	1,616.65	48.91	22,531.45
Parys Mt	597.07	54,138.1	248.98	482.96	10.27	2.73	40.94	1,442.70
Broekpolder	53,814.7	26,300.1	828.78	153.75	38.35	10.52	279.85	222.25
Oostabtspolder	38,749.6	23,553.4	446.36	85.92	30.48	9.23	153.60	118.06
Spieringpolder	31,800.1	24,630.6	731.28	88.88	33.14	5.02	95.85	201.28
Neckar sludge	54,146.0	21,837.8	521.03	205.25	52.45	27.68	382.49	155.36
WES reference	1,156.6	10,626.9	68.90	8.38	13.83	< 0.13	21.19	8.98

Table 3.3 DTPA - extractable metal concentrations in the experimental soils and sediments ($\mu g\ g^{-1}$, oven dry weight)

SOIL/SEDIMENT	Ca	Fe	ZN	Cu	Ni	Cd	Cr	Pb
Frongoch	89.23	147.03	3.96	5.83	0.70	0.11	0.04	21.87
Ystwyth	< 0.06	85.34	183.78	9.42	0.90	1.06	< 0.03	525.04
Halkyn Mt	750.89	16.36	331.77	45.18	3.06	2.33	0.04	195.20
Shipham	61.46	0.56	636.96	1.50	0.26	25.81	0.04	64.76
Parys Mt	< 0.06	518.04	0.86	9.22	0.14	0.02	0.04	1.68
Broekpolder	1,929.75	206.95	239.15	50.80	2.78	5.56	0.10	20.28
Oostabtspolder	2,013.52	227.67	125.84	29.50	2.58	3.47	0.08	15.51
Spieringpolder	1,372.11	84.52	110.36	27.12	0.95	2.11	0.06	40.38
Neckar sludge	2,152.30	174.06	128.63	79.44	4.12	13.99	0.12	33.18

Table 3.4 Ratio of DTPA extractable : Total heavy metal concentrations in experimental soils and sediments

SOIL/SEDIMENT	Zn	Cu	Ni	Cd	Cr	Pb
Frongoch	0.034	0.255	0.027	0.121	0.001	0.288
Ystwyth	0.233	0.271	0.022	0.351	<0.001	0.364
Halkyn Mt	0.240	0.378	0.052	0.685	0.001	0.207
Shipham	0.004	0.007	0.010	0.016	0.001	0.003
Parys Mt	0.003	0.019	0.014	0.007	0.001	0.001
Broekpolder	0.289	0.330	0.072	0.529	<0.001	0.091
Oostabtspolder	0.270	0.343	0.085	0.376	0.001	0.131
Spieringpolder	0.151	0.305	0.029	0.420	0.001	0.201
Neckar sludge	0.247	0.387	0.079	0.505	<0.001	0.214

Table 3.5 Recovery and weight change of earthworms over the experimental period (Expressed as a percentage of the weight of earthworms added to each pot at the start of the experiment).

	% RECOVERY	DAY 15	DAY 28	DAY 56
FRONGOCH	93.6			
E. foetida		-2.6	-8.1	-11.4
L. terrestris		-7.4	-6.0	-14.6
A. longa		-17.8	-3.0	-26.7
A. caliginosa		+4.2	+8.2	-2.2
A. chlorotica		+3.4	+7.4	-7.7
L. rubellus			-20.4	
YSTWYTH	93.3			
E. foetida		-10.5	-13.9	-28.2
L. terrestris		+6.8	-12.7	-17.4
A. longa		+2.1	+5.2	-30.4
A. chlorotica		0	-7.7	-24.0
HALKYN MT.	93.3			
E. foetida		-5.1	-15.0	-26.3
L. terrestris		-2.9	-4.9	-19.8
A. longa		-4.3	-14.8	-40.7
SHIPHAM	86.5			
E. foetida		-16.7	-30.0	-44.7
L. terrestris		-11.8	-22.7	-48.0
A. longa		-24.8	-38.4	-49.0
A. caliginosa		-15.9	-31.1	-33.0
PARYS MT.	97.5			
E. foetida		+12.8	+11.1	-5.6
L. terrestris		+4.5	-5.1	-42.7
A. longa		+18.7	-8.7	-39.8
B-POLDER	92.5			
E. foetida		-7.5	-12.9	-29.3
L. terrestris		-7.5	-12.9	-29.3
A. longa		-17.7	-17.6	-35.0
L. rubellus			-33.2	
A-POLDER	82.0			
E. foetida		-5.4	-15.0	-35.7
L. terrestris			-13.6	
A. longa		-7.7	-24.2	-45.7
A. caliginosa			-18.6	

Table 3.5 (continued)

	% RECOVERY	DAY 15	DAY 28	DAY 56
S-POLDER	93.9			
E. foetida		-10.8	-8.1	-24.4
L. terrestris		+2.2	-9.4	-12.4
A. longa			-34.3	
A. caliginosa		-17.1	-22.7	-32.6
N-SLUDGE	93.4			
E. foetida		-5.4	-7.9	-23.7
L. terrestris			-14.9	
A. longa		-27.9	-37.3	-50.4
A. chlorotica		-24.0	-32.0	-50.0
WES REF -				
E. foetida		-12.2	-17.6	-28.20

(-ve = weight loss; +ve = weight gain)

Table 3.6 Mean heavy metal contentrations in earthworm tissue at the start of the experiment and after 15, 28 and 56 days in the soils and sediments ($\mu g\ g^{-1}$, dry weight)

Table 3.6a ELEMENT - ZINC

	INITIAL	DAY 15	DAY 28	DAY 56
FRONGOCH				
E. foetida	129	107	146	101
L. terrestris	457	692	615	584
A. longa	686	884	824	840
A. caliginosa	478	470	596	581
A. chlorotica	333	284	314	316
L. rubellus	454	-	688	-
YSTWYTH				
E. foetida	129	87	156	104
L. terrestris	457	578	839	977
A. longa	686	852	770	1362
A. chlorotica	333	396	504	773
HALKYN MT.				
E. foetida	129	174	192	190
L. terrestris	457	428	816	853
A. longa	686	899	972	1112
SHIPHAM				
E. foetida	129	4526	2055	5401
L. terrestris	457	9643	3359	8313
A. longa	686	-ve	1687	2479
A. caliginosa	478	-ve	5633	2966
PARYS MT.				
E. foetida	129	46	85	61
L. terrestris	457	329	582	797
A. longa	686	570	630	716
B-POLDER				
E. foetida	129	174	173	140
L. terrestris	457	686	468	494
A. longa	686	908	850	-
L. rubellus	454	-	770	-
A-POLDER				
E. foetida	129	160	158	137
L. terrestris	457	-	522	-
A. longa	686	702	894	847
A. caliginosa	478	420	462	

Table 3.6 (continued) ELEMENT - ZINC

	INITIAL	DAY 15	DAY 28	DAY 56
S-POLDER				
E. foetida	129	158	119	125
L. terrestris	457	769	516	473
A. longa	686	-	816	-
A. caliginosa	478	-	526	459
N-SLUDGE				
E. foetida	129	148	124	132
L. terrestris	457	-	674	-
A. longa	686	564	752	638
A. chlorotica	333	277	250	285

Table 3.6 (b) ELEMENT - COPPER

	INITIAL	DAY 15	DAY 28	DAY 56
FRONGOCH				
E. foetida	17.2	9.1	10.0	10.7
L. terrestris	12.4	6.5	6.0	10.0
A. longa	17.3	9.0	6.9	5.2
A. caliginosa	7.9	5.6	5.3	5.9
A. chlorotica	6.7	6.0	6.7	7.2
L. rubellus	7.8	-	7.9	
YSTWYTH				
E. foetida	17.2	9.8	12.4	10.7
L. terrestris	12.4	4.9	5.6	11.4
A. longa	17.3	16.0	13.5	8.2
A. chlorotica	6.7	7.1	6.4	8.5
HALKYN MT.				
E. foetida	17.2	29.0	36.2	46.3
L. terrestris	12.4	28.0	25.3	25.0
A. longa	17.3	31.0	29.4	26.5
SHIPHAM				
E. foetida	17.2	7.8	-ve	35.1
L. terrestris	12.4	-ve	-ve	16.6
A. longa	17.3	-ve	8.4	8.32
A. caliginosa	7.9	-ve	8.0	11.7
PARYS MT.				
E. foetida	17.2	-ve	15.6	30.0
L. terrestris	12.4	-ve	-ve	27.0
A. longa	17.3	-ve	-ve	16.9
B-POLDER				
E. foetida	17.2	41.3	37.6	38.6
L. terrestris	12.4	25.1	23.5	29.5
A. longa	17.3	25.1	33.5	-
L. rubellus	7.8	-	21.4	-
A-POLDER				
E. foetida	17.2	25.2	23.5	20.9
L. terrestris	12.4	-	19.2	-
A. longa	17.3	27.4	24.1	17.4
A. caliginosa	7.9	-	15.7	-

Table 3.6 (b) (continued) ELEMENT - COPPER

	INITIAL	DAY 15	DAY 28	DAY 56
S-POLDER				
E. foetida	17.2	24.8	22.4	22.5
L. terrestris	12.4	18.7	11.2	12.7
A. longa	17.3	-	14.8	-
A. caliginosa	7.9	27.3	10.4	12.7
N-SLUDGE				
E. foetida	17.2	57.8	55.3	50.2
L. terrestris	12.4	-	37.9	-
A. longa	17.3	45.3	44.3	46.0
A. chlorotica	6.7	23.6	18.8	30.8

Table 3.6 (c) ELEMENT - NICKEL

	INITIAL	DAY 15	DAY 28	DAY 56
FRONGOCH				
E. foetida	<0.75	0.79	3.66	1.68
L. terrestris	0.92	0.82	0.40	4.33
A. longa	1.53	2.27	0.85	-ve
A. caliginosa	0.84	0.40	0.89	3.86
A. chlorotica	1.09	0.75	1.41	4.76
L. rubellus	1.46	-	5.13	-
YSTWYTH				
E. foetida	<0.75	-ve	2.32	-ve
L. terrestris	0.92	-ve	-ve	-ve
A. longa	1.53	1.36	-ve	-ve
A. chlorotica	1.09	0.90	-ve	2.15
HALKYN MT.				
E. foetida	<0.05	0.91	2.55	3.57
L. terrestris	0.92	3.35	1.91	1.59
A. longa	1.53	2.56	1.78	-ve
SHIPHAM				
E. foetida	<0.75	-ve	-ve	8.22
L. terrestris	0.92	-ve	-ve	1.05
A. longa	1.53	-ve	-ve	0.29
A. caliginosa	0.84	-ve	1.48	3.01
PARYS MT.				
E. foetida	<0.75	-ve	-ve	6.83
L. terrestris	0.92	3.66	2.22	0.95
A. longa	1.53	0.95	0.86	0.63
B-POLDER				
E. foetida	<0.75	3.65	3.11	5.76
L. terrestris	0.92	4.75	1.01	2.16
A. longa	1.53	1.21	2.95	-
L. rubellus	1.46	-	5.25	-
A-POLDER				
E. foetida	<0.75	2.34	2.44	9.86
L. terrestris	0.92	-	3.75	-
A. longa	1.53	5.79	4.61	1.10
A. caliginosa	0.84	-	2.24	-

Table 3.6 (c) (continued) ELEMENT - NICKEL

	INITIAL	DAY 15	DAY 28	DAY 56
S-POLDER				
E. foetida	<0.75	2.62	-ve	3.77
L. terrestris	0.92	3.69	0.80	0.77
A. longa	1.53	-	1.20	-
A. caliginosa	0.84	1.54	1.01	2.68
N-SLUDGE				
E. foetida	<0.75	4.67	3.12	7.13
L. terrestris	0.92	-	3.10	-
A. longa	1.53	3.32	3.48	2.39
A. chlorotica	1.09	2.19	1.23	8.25

Table 3.6 (d) ELEMENT - CADMIUM

	INITIAL	DAY 15	DAY 28	DAY 56
FRONGOCH				
E. foetida	2.14	-ve	0.08	1.00
L. terrestris	5.24	4.71	7.00	2.99
A. longa	5.42	5.35	11.3	7.68
A. caliginosa	10.5	7.36	10.2	9.13
A. chlorotica	7.74	6.78	8.45	8.90
L. lrubellus	3.02	-	2.49	-
YSTWYTH				
E. foetida	2.14	-ve	0.53	1.27
L. terrestris	5.24	4.07	6.20	9.84
A. longa	5.42	4.55	7.71	26.1
A. chlorotica	7.74	7.05	13.8	19.9
HALKYN MT.				
E. foetida	2.14	3.35	6.23	8.70
L. terrestris	5.24	8.33	14.8	19.5
A. longa	5.42	14.1	14.8	16.9
SHIPHAM				
E. foetida	2.14	52.4	139	196
L. terrestris	5.24	71.0	127	354
A. longa	5.42	42.9	89.4	138
A. caliginosa	10.5	-ve	142	150
PARYS MT.				
E. foetida	2.14	-ve	2.10	1.53
L. terrestris	5.24	-ve	1.01	8.99
A. longa	5.42	2.01	4.32	12.3
B-POLDER				
E. foetida	2.14	4.97	6.86	11.8
L. terrestris	5.24	13.1	9.28	13.5
A. longa	5.42	12.0	16.9	-
L. rubellus	3.02	-	8.17	-
A-POLDER				
E. foetida	2.14	3.79	5.33	12.6
L. terrestris	5.24	-	11.0	-
A. longa	5.42	12.6	12.0	23.2
A. caliginosa	10.5	-	17.0	-

Table 3.6 (d) (continued) ELEMENT - CADMIUM

	INITIAL	DAY 15	DAY 28	DAY 56
S-POLDER				
E. foetida	2.14	2.57	3.30	4.92
L. terrestris	5.24	7.45	7.00	5.71
A. longa	5.42	-	12.7	-
A. caliginosa	10.5	15.0	17.3	16.6
N-SLUDGE				
E. foetida	2.14	10.6	14.3	24.7
L. terrestris	5.24	-	16.1	-
A. longa	5.42	17.0	21.1	23.0
A. chlorotica	7.74	10.6	13.4	19.0

Table 3.6 (e) ELEMENT - CHROMIUM

	INITIAL	DAY 15	DAY 28	DAY 56
FRONGOCH				
E. foetida	2.58	6.56	4.65	9.64
L. terrestris	1.34	4.67	6.56	17.9
A. longa	2.18	5.87	5.18	4.76
A. caliginosa	3.01	2.12	4.09	8.50
A. chlorotica	2.98	2.93	5.63	9.37
L. rubellus	4.38	-	14.8	-
YSTWYTH				
E. foetida	2.58	1.69	3.34	7.28
L. terrestris	1.34	2.30	7.08	10.4
A. longa	2.18	2.51	16.4	4.80
A. chlorotica	2.98	6.25	4.87	3.47
HALKYN MT.				
E. foetida	2.58	7.42	7.13	11.8
L. terrestris	1.34	7.48	11.9	12.2
A. longa	2.18	2.40	9.64	2.56
SHIPHAM				
E. foetida	2.58	-ve	-ve	6.09
L. terrestris	1.34	-ve	-ve	-ve
A. longa	2.18	-ve	-ve	1.23
A. caliginosa	3.01	-ve	2.69	2.16
PARYS MT.				
E. foetida	2.58	-ve	-ve	2.19
L. terrestris	1.34	-ve	-ve	2.30
A. longa	2.18	-ve	-ve	9.34
B-POLDER				
E. foetida	2.58	22.6	16.5	11.4
L. terrestris	1.34	29.4	14.2	17.9
A. longa	2.18	10.5	19.2	-
L. rubellus	4.38	-	30.3	-
A-POLDER				
E. foetida	2.58	23.1	18.2	11.8
L. terrestris	1.34	-	32.7	-
A. longa	2.18	29.5	32.3	6.14
A. caliginosa	3.01	-	16.6	-

Table 3.6 (e) (continued) ELEMENT - CHROMIUM

	INITIAL	DAY 15	DAY 28	DAY 56
S-POLDER				
E. foetida	2.58	10.1	3.17	5.45
L. terrestris	1.34	9.38	5.54	5.65
A. longa	2.18	-	4.23	-
A. caliginosa	3.01	10.1	6.50	5.08
N-SLUDGE				
E. foetida	2.58	35.3	19.5	25.1
L. terrestris	1.34	-	39.0	-
A. longa	2.18	19.1	10.6	12.2
A. chlorotica	2.98	13.2	6.57	7.57

Table 3.6 (f) ELEMENT - LEAD

	INITIAL	DAY 15	DAY 28	DAY 56
FRONGOCH				
E. foetida	2.64	3.91	2.21	4.07
L. terrestris	7.99	4.71	4.69	16.1
A. longa	19.7	16.0	17.0	8.30
A. caliginosa	10.1	5.95	8.58	13.6
A. chlorotica	6.41	6.55	9.82	16.9
L. rubellus	4.52	-	22.1	-
YSTWYTH				
E. foetida	2.64	81.7	310	572
L. terrestris	7.99	-ve	61.6	188
A. longa	19.7	234	261	350
A. chlorotica	6.41	126	223	487
HALKYN MT.				
E. foetida	2.64	18.8	41.9	44.6
L. terrestris	7.99	40.5	47.4	70.5
A. longa	19.7	151	98.9	26.6
SHIPHAM				
E. foetida	2.64	10289	5432	10112
L. terrestris	7.99	6708	7319	20636
A. longa	19.7	8770	8559	3982
A. caliginosa	10.1	5434	5406	3739
PARYS MT.				
E. foetida	2.64	-ve	-ve	18.3
L. terrestris	7.99	-ve	-ve	218
A. longa	19.7	-ve	-ve	39.9
B-POLDER				
E. foetida	2.64	11.7	11.5	5.45
L. terrestris	7.99	10.2	8.26	6.45
A. longa	19.7	16.2	25.8	-
L. rubellus	4.52	-	12.2	-
A-POLDER				
E. foetida	2.64	10.6	4.86	1.82
L. terrestris	7.99	-	15.1	-
A. longa	19.7	41.1	21.7	41.9
A. caliginosa	10.1	-	18.9	-

Table 3.6 (f) (continued) ELEMENT - LEAD

	INITIAL	DAY 15	DAY 28	DAY 56
S-POLDER				
E. foetida	2.64	12.6	2.89	2.49
L. terrestris	7.99	16.3	5.01	15.5
A. longa	19.7	-	20.7	-
A. caliginosa	10.1	19.6	10.9	10.9
N-SLUDGE				
E. foetida	2.64	7.89	4.51	10.0
L. terrestris	7.99	-	11.7	-
A. longa	19.7	23.4	12.3	13.4
A. chlorotica	6.41	3.76	4.54	4.38

Table 3.7 Part 1
Percent variance accounted for by the linear regression between each species of earthworm and the total/DTPA extractable metal in the soil. All data $\log_e$.

	DAY 15 SAMPLE		DAY 28 SAMPLE		DAY 56 SAMPLE	
ELEMENT/ EARTHWORM SP.	Total metal	DTPA metal	Total metal	DTPA metal	Total metal	DTPA metal
ZINC						
E. foetida	75.3	32.9	85.5	24.9	90.4	25.5
L. terrestris	67.8	17.1	64.5	10.0	64.6	0.8
A. longa	0.5	7.2	55.4	25.3	-	-
A. caliginosa	8.5	8.5	63.3	11.6	65.8	28.0
A. chlorotica	19.0	9.0	1.7	-	24.3	11.9
CADMIUM						
E. foetida	78.3	77.6	77.6	46.6	80.9	64.7
L. terrestris	76.1	58.6	68.8	56.2	86.3	39.0
A. longa	18.8	52.8	67.2	53.3	86.5	52.9
A. caliginosa	68.2	68.2	95.1	69.7	97.0	83.3
A. chlorotica	57.7	51.1	32.2	43.7	43.9	57.8
LEAD						
E. foetida	84.9	4.0	77.4	23.9	75.4	22.8
L. terrestris	84.8	1.6	77.5	2.9	87.6	-
A. longa	91.9	15.4	88.2	8.1	75.7	9.6
A. caliginosa	96.0	69.9	86.0	28.4	88.3	55.2
A. chlorotica	39.3	37.2	83.0	89.5	81.2	87.6

- = residual variance exceeds variance of Y variate

Table 3.7 Part 2
Percent variance accounted for by the linear regression between each species of earthworm and the total/DTPA extractable metal in the soil. Data NOT logged.

	DAY 15 SAMPLE		DAY 28 SAMPLE		DAY 56 SAMPLE	
ELEMENT/ EARTHWORM SP.	Total metal	DTPA metal	Total metal	DTPA metal	Total metal	DTPA metal
COPPER						
E. foetida	40.0	95.5	7.2	96.1	3.6	62.6
L. terrestris	35.5	36.3	77.7	83.0	10.0	27.1
A. longa	-	73.3	10.3	88.1	7.6	94.3
A. caliginosa	35.6	35.6	-	57.1	22.1	3.6
A. chlorotica	99.2	99.2	78.5	78.6	88.8	88.8
CHROMIUM						
E. foetida	79.5	84.2	41.0	45.8	28.8	25.4
L. terrestris	29.8	29.4	38.1	44.5	2.0	-
A. longa	28.4	43.8	0.7	4.8	39.1	34.9
A. caliginosa	20.8	20.8	72.7	73.1	-	-
A. chlorotica	84.2	76.9	-	-	7.4	14.0
NICKEL						
E. foetida	1.3	29.5	-	-	-	-
L. terrestris	-	-	-	1.1	-	-
A. longa	-	12.0	11.2	50.8	20.7	47.1
A. caliginosa	-	-	13.9	72.5	0.5	-
A. chlorotica	81.5	87.3	-	-	0.2	20.1

- = residual variance exceeds variance of Y variate.

Relationship between zinc concentrations in earthworms and soil

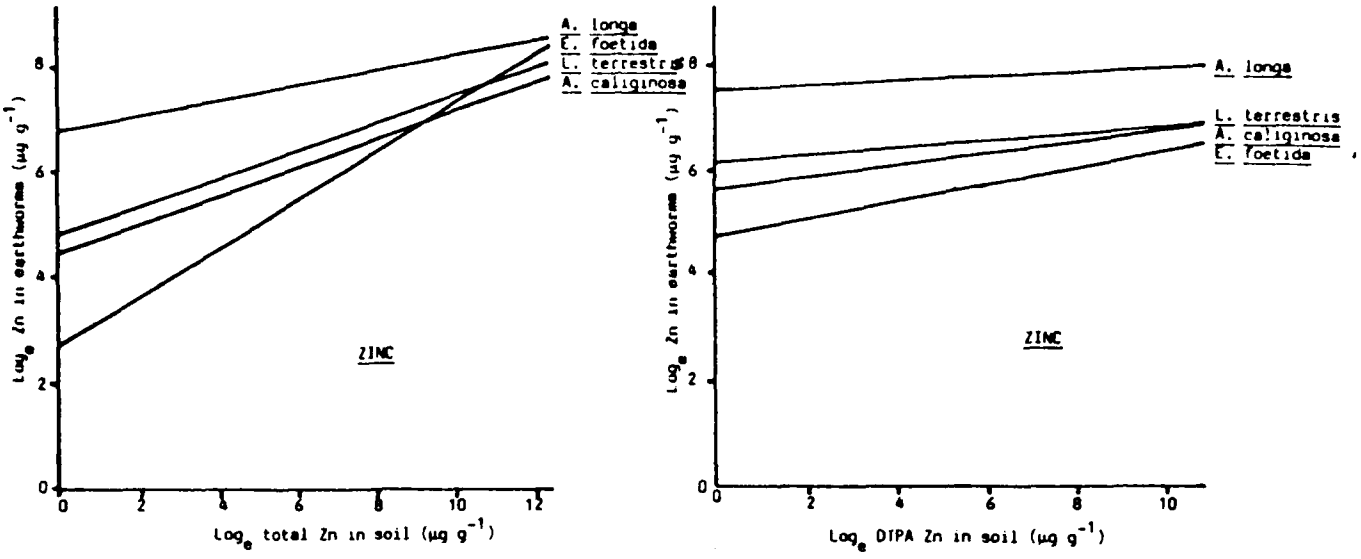


Figure 3.1

Relationship between Copper concentrations in earthworms and soil

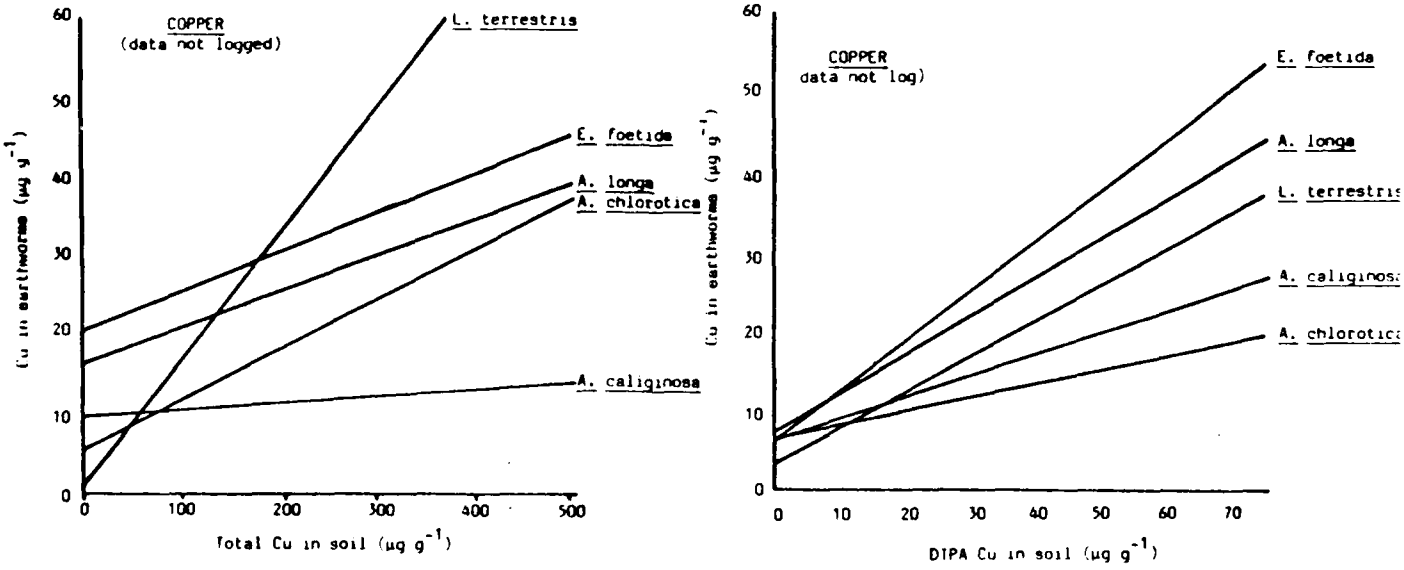


Figure 3.2

Relationship between Cadmium concentrations in earthworms and soil.

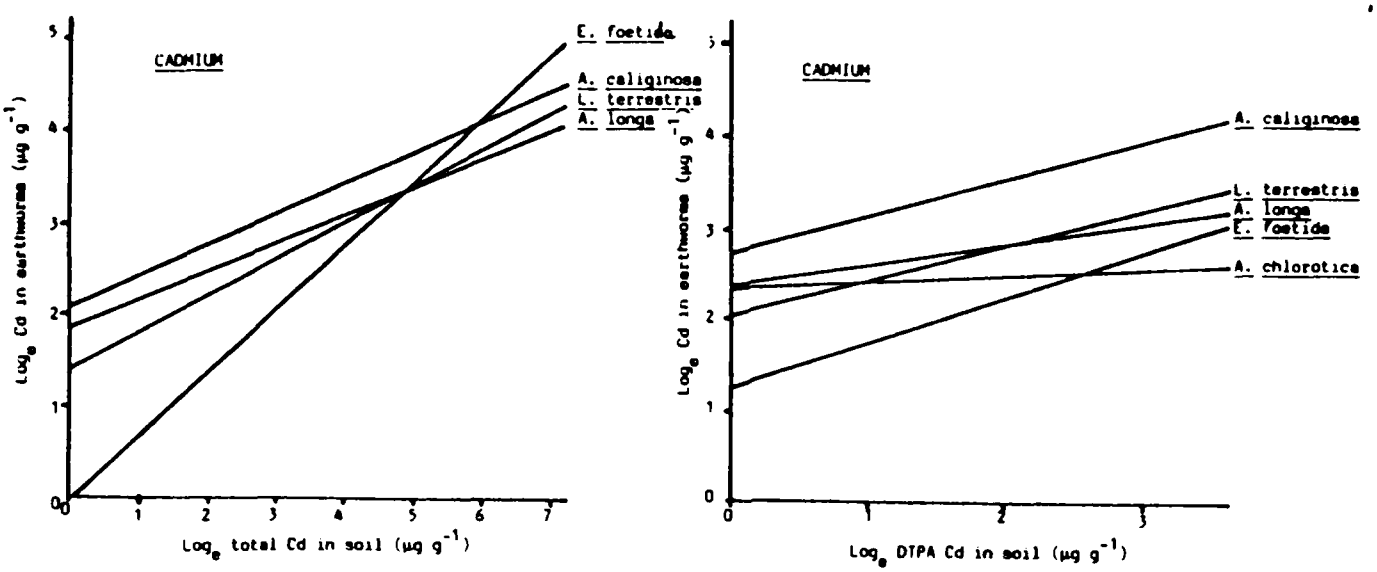


Figure 3.3

Relationship between Lead concentrations in earthworms and soil.

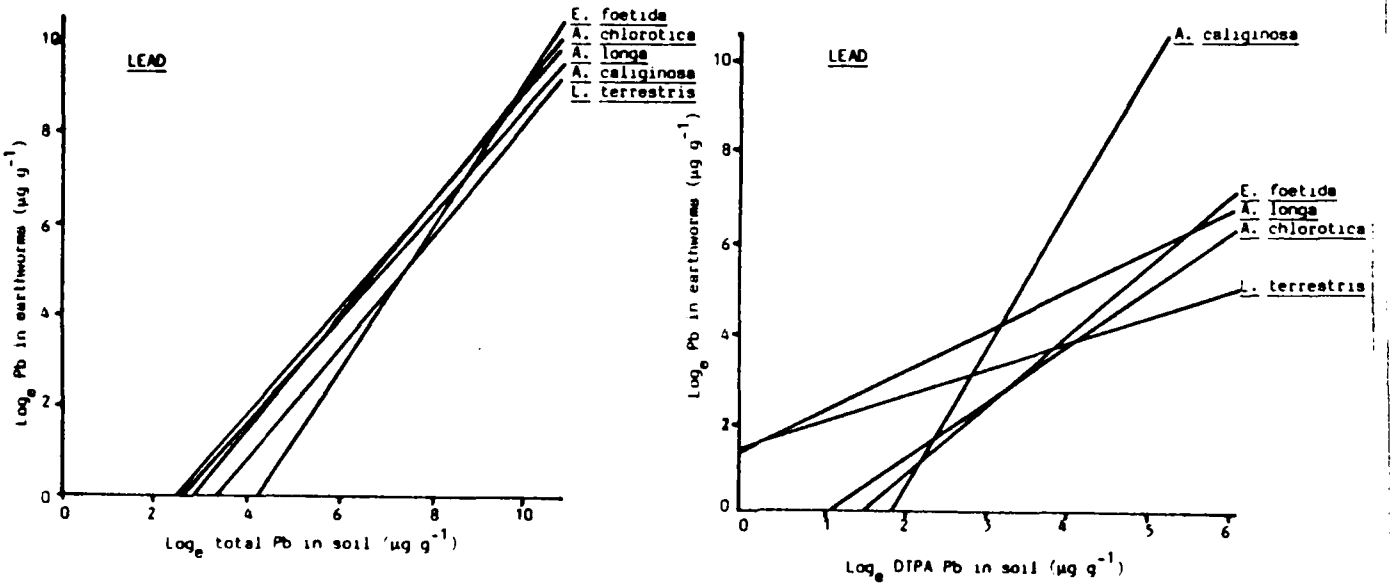


Figure 3.4

3.5 Relationship between Metal Concentrations in *E. foetida* and the Field Species of Earthworm

The concentration of each of the elements Zn, Cu, Ni, Cd, Cr and Pb measured in *E. foetida* after 15, 28 and 56 days in the soils was correlated with the concentration of the corresponding element measured in each of the field species at the respective sampling time. The co-efficients of correlation are given in Table 3.8.

In the majority of combinations a good correlation was observed between metal concentrations in *E. foetida* and metal concentrations in field species of earthworm. A consistently close linear relationship was evident between Pb concentrations in *E. foetida* and each of the four field species. Cu concentrations in *E. foetida* were also well correlated with Cu concentrations in each of the four field species. Higher co-efficients of correlation were recorded between Zn and Cd concentrations in *E. foetida* and those in *L. terrestris*, *A. longa* and *A. caliginosa* compared with *E. foetida* and *A. chlorotica*.

Some negative correlations were recorded for Ni concentrations in earthworms and generally there was very little relationship between Ni concentration in *E. foetida* and the other earthworm species. A relatively poor relationship was also evident between Cr concentrations in *E. foetida* and *A. longa*.

Generally, closer correlation between metal concentrations in *E. foetida* and the field species was observed after 15 and 28 days in the experimental soils compared with earthworms sampled after a 56-day experimentalperiod (Table 3.8).

The linear relationship between Zn, Cu, Cd and Pb concentrations in *E. foetida* and each of the field species after 28 days in the experimental soils is illustrated in Figure 3.5. These figures clearly demonstrate that increasing metal concentrations in *E. foetida* are linearly correlated with increasing metal levels in each of the field species (with the sole exception of Zn concentrations in *E. foetida* and *A. chlorotica*).

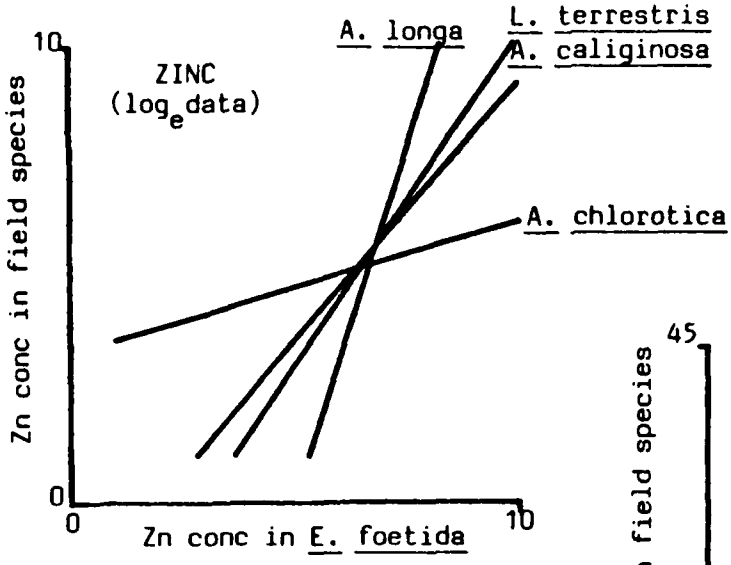
Table 3.8 Co-efficients of correlation between heavy metal levels measured in *E. foetida* and each of the field species after 15, 28 and 56 days

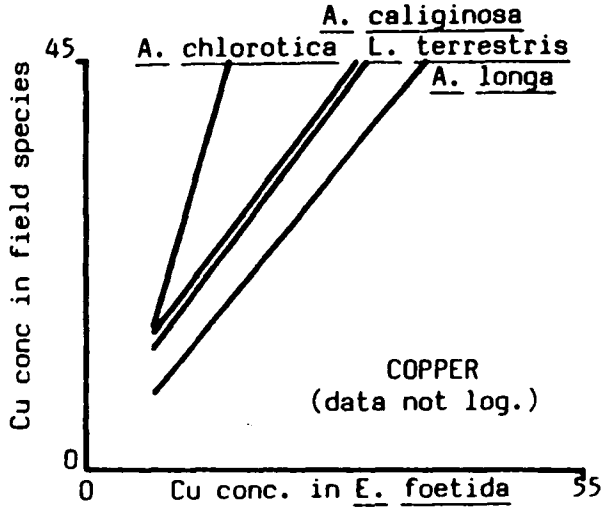
E. foetida	*L. terrestris*	*A. longa*	*A. caliginosa*	*A. chlorotica*
ZINC				
Day 15	0.966	0.109	-	-0.822
Day 28	0.931	0.968	0.990	0.893
Day 56	0.923	0.849	0.986	-0.492
COPPER				
Day 15	0.909	0.908	-	0.999
Day 28	0.980	0.974	0.895	0.998
Day 56	0.808	0.805	0.778	0.999
NICKEL				
Day 15	0.806	0.012	-	-
Day 28	-0.713	-0.605	-	-
Day 56	-0.656	-0.198	-0.537	-
CADMIUM				
Day 15	0.979	0.967	-	0.996
Day 28	0.836	0.885	0.982	0.570
Day 56	0.938	0.897	0.999	0.508
CHROMIUM				
Day 15	0.985	0.228	-	0.897
Day 28	0.892	0.607	0.963	0.925
Day 56	0.817	0.509	0.814	0.337
LEAD				
Day 15	0.993	0.957	0.999	0.946
Day 28	0.951	0.981	0.999	0.846
Day 56	0.923	0.933	0.999	0.915

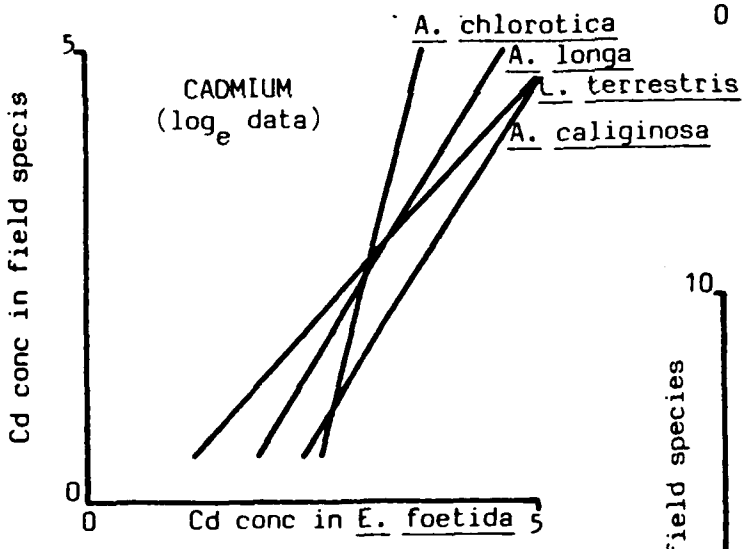
Log_e data used for correlations involving Zn; Cd and Pb
Normal data used for correlations involving Cu; Ni and Cr.
- = insufficient data available for calculation.

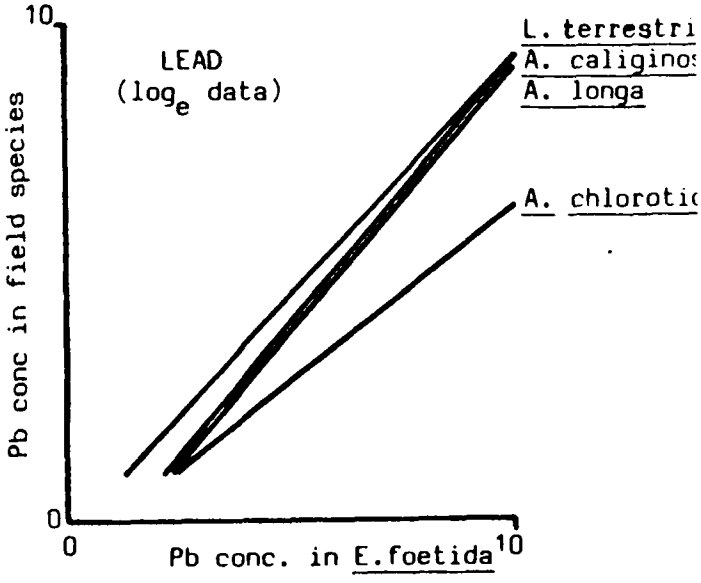
FIGURE 3.5

Relationship between metal concentrations in E. foetida and the field species of earthworm









4. DISCUSSION

4.1 Correlation between Earthworm Tissue and Soil Metal Concentrations

Uptake of heavy metals by organisms from their feed or substrate is influenced by numerous variables of which the absolute concentration of the element within the feed/substrate is only one. Many other factors such as pH; cation exchange capacity; Ca ion concentration; soil particle size, clay content and organic matter content have been demonstrated to influence the uptake of metals into plant and animal tissues. Furthermore, the chemical form of each element as well as its concentration relative to other elements present will influence the extent of antagonistic and synergistic interactions which also affect metal uptake (Nicholas and Egan, 1975).

Soil physical and chemical properties have been clearly demonstrated to influence heavy metal uptake into earthworm tissue (Ireland, 1979; Ma, 1982; 1983; 1984; Streit and Jaggy, 1983). Therefore, in comparing metal uptake by earthworms grown in soils of different heavy metal composition (Table 3.2) it is likely that the physico-chemical variables (Table 3.1) will also influence metal uptake and may obscure trends of metal uptake and correlations between earthworm tissue and soil metal concentrations.

Despite these additional variables some good linear correlations between earthworm tissue and soil metal concentrations emerged in the present study (Table 3.7). For some elements e.g. Cu a close linear relationship was found between earthworm tissue concentrations and soil DTPA extractable concentrations of each element, while for Zn, Cd and particularly Pb a greater percentage of the variance was accounted for by the linear relationship between earthworm tissue and soil total ($HNO_3/HClO_4$) metal concentrations. To some extent this may have been a reflection of the chemical extractant used. The extraction procedure using DTPA was developed to assess possible deficiency of Zn, Fe, Mn and Cu in the soil. By contrast, soils used in the present study contained elevated levels of these elements (Table 3.2). However, the method may be expected to give a good indication of the availability of the elements for which it was developed. Zn and Cd are known to behave in a similar manner in the soil and DTPA may also be used to estimate Cd availability in the soil. However, DTPA is rarely used to assess Pb availability due to the poor results obtained (McGrath pers. comm.*). Other soil extractants may provide a better indication of the Pb fraction available for uptake into plant and animal tissues.

Earthworms of all species studied appeared to be poor indicators of the level of Zn (total and DTPA extractable) in the soil (Table 3.7, Figure 3.1). This may be a result of the high base line levels of Zn measured in earthworms at the start of the experiment (Table 3.6). These levels did not arise due to the earthworms being collected from soil polluted by Zn (Table 2.2) and previous studies have also shown earthworms from unpolluted sites to contain high base line concentrations of Zn (Ma 1982a, b). Zn is known to be an essential element for growth and tissue levels of Zn in earthworms appear to be regulated (Ireland, 1979; Beyer, 1981). These characteristics would reduce the usefulness of earthworms as indicators of Zn availability and the results of this study suggest that although there is a positive relationship between earthworm and soil Zn concentrations, for all species except *E. foetida* a relatively small

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percentage of the variance is accounted for by this relationship. It is interesting to note that of all species studied, *E. foetida* contained the lowest base-line concentration of Zn (Table 3.6) which may explain the better response to soil Zn concentrations. Some previous research using field species of earthworm has also suggested that there is little significant relationship between earthworm and soil Zn concentrations (Roberts and Johnson, 1978; Martin and Coughtrey, 1982).

Cu is also an essential element for growth and tissue levels appear to be regulated by earthworms (Ireland 1979). However, concentrations of Cu in earthworm tissue have been shown to increase with increasing Cu contamination as a result of sludge and pig-waste application to soil (Helmke *et al*; 1979; Curry and Cotton, 1982). Similar results were reported in the present study with strong positive linear correlations between earthworm and soil DTPA extractable Cu concentrations (Table 3.7, Figure 3.4). This suggests that earthworms would be good indicators of Cu availability in the soil.

Uptake and accumulation of Cd by earthworms is well documented (Andersen, 1979; Helmke *et al.*, 1979; Beyer, 1981; Ireland, 1983) and higher concentrations of this non-essential element have been frequently recorded in earthworm tissue compared with the surrounding soil/substrate. Results of the present study also showed a strong positive linear correlation between earthworm and soil total Cd concentrations and a fairly good relationship between earthworm and soil DTPA extractable Cd concentrations (Table 3.7, Figure 3.2). This suggests that earthworms provide a good indication of levels of Cd available for uptake from the soil.

Some disagreement exists in the literature regarding uptake of the non-essential element Pb by earthworms in relation to soil Pb levels (Beyer, 1981; Ireland, 1983). In making comparisons with data reported in the literature the method of depuration of soil from the earthworm gut should be considered (for further discussion see Stafford and McGrath; Appendix 2). Results of the present experiment clearly showed that earthworms of all species were good indicators of the total Pb concentration in the soil (Table 3.7; Figure 3.3). If this effect were due to the presence of soil within the earthworm gut then equally good correlations would be expected for all elements. However, the poor correlations observed for Zn, Cr and Ni (Table 3.7) confirm that this pattern of correlation truly represents uptake of Pb into earthworm tissue from the surrounding soil.

In summary: it would appear from the data presented that earthworm tissue concentrations of the elements Cu, Pb and Cd represent a good indication of increasing concentrations of these elements in the soil. Cu and Cd concentrations in earthworm tissue were linearly related to soil DTPA extractable Cu and Cd levels while Pb and Cd concentrations were related to soil total metal levels. The strength of linear relationship between earthworm tissue and soil Zn concentrations varied considerably between species with generally poor correlation co-efficients. This suggested that earthworms, with the possible exception of *E. foetida*, would not provide a good indication of the availability of Zn in the soil. The strength of correlation between earthworm and soil metal concentrations did not increase with increasing experimental period which suggests that no additional benefit would be gained from conducting an earthworm bioassay for periods greater than 28 days.

4.2 Comparative Metal Uptake between Earthworm Species

Inter specific differences in heavy metal concentrations of earthworms from the same sites have been reported (Ireland and Richards, 1977; Ireland, 1979; Ash and Lee, 1980). These differences could result from behavioural and feeding differences; differences in uptake mechanisms and metabolism of various elements, as has been suggested for Ca (Piearce, 1972), and/or from differences in tolerance to levels of heavy metals within the tissue (Ireland, 1983). Although absolute concentrations of metals within the earthworm tissue may differ between species, a close, positive relationship in metal uptake between species, as observed in the present experiment (Table 3.8), would suggest that one species can serve to indicate metal availability to the others. Results given in Table 3.8 correlating uptake of metals by *E. foetida* with uptake by the field species, held under the same environmental conditions, show consistently high correlation co-efficients for the elements Zn, Cu, Cd and Pb, with the possible exception of the relationship between *E. foetida* and *A. chlorotica*.

For maximum protection of the ecosystem the surrogate species selected for use in laboratory bioassays should represent the 'worst case' of metal uptake by the group. Slopes of the graphs in Figures 3.1 - 3.4 indicate that of the species studied, a higher rate of uptake of heavy metals by *E. foetida* with increasing soil metal concentrations was observed compared with the four field species of earthworm. In addition, results of correlations between earthworm and soil metal levels, given in Table 3.8, also show consistently high correlations between *E. foetida* and soil metal levels.

In summary: The results demonstrate that uptake of the metals Zn, Cu, Cd and Pb had a strong, positive linear correlation with uptake of these elements by the four field species of earthworm tested. The use of *E. foetida* as a surrogate species in laboratory bioassays was favoured since this species showed consistently good linear correlation between earthworm and soil metal levels and also exhibited a greater response in tissue metal levels to increasing concentrations of elements in the soil.

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APPENDIX I

Table 1: Mean heavy metal concentrations in earthworm samples (earthworm tissue plus soil within the earthworm gut) after 15, 28 and 56 days in the soils/sediments ($\mu g\ g^{-1}$, dry weight).

ELEMENT - ZINC

	DAY 15	DAY 28	DAY 56
FRONGOCH			
E. foetida	110	136	106
L. terrestris	458	639	426
A. longa	563	609	609
A. caliginosa	329	406	415
A. chlorotica	229	253	252
L. rubellus	-	402	-
YSTWYTH			
E. foetida	378	373	306
L. terrestris	699	808	889
A. longa	816	784	1183
A. chlorotica	522	601	775
HALKYN MT.			
E. foetida	618	548	517
L. terrestris	911	1098	1075
A. longa	1149	1127	1129
SHIPHAM			
E. foetida	34253	20024	29241
L. terrestris	81372	72080	60185
A. longa	57928	39855	22329
A. caliginosa	47454	16218	13405
PARYS MT.			
E. foetida	139	160	135
L. terrestris	283	393	603
A. longa	408	495	647
B-POLDER			
E. foetida	313	285	245
L. terrestris	730	515	519
A. longa	890	844	-
L. rubellus	-	791	-
A-POLDER			
E. foetida	229	213	166
L. terrestris	-	503	-
A. longa	619	776	832
A. caliginosa	-	463	-

ELEMENT - ZINC (Continued)

	DAY 15	DAY 28	DAY 56
S-POLDER			
E. foetida	231	156	185
L. terrestris	745	585	556
A. longa	-	790	-
A. caliginosa	470	542	479
N-SLUDGE			
E. foetida	241	184	195
L. terrestris	-	620	-
A. longa	551	700	624
A. chlorotica	301	263	299

ELEMENT - COPPER

	DAY 15	DAY 28	DAY 56
FRONGOCH			
E. foetida	14.3	14.6	14.7
L. terrestris	13.4	13.6	14.6
A. longa	14.8	12.5	10.8
A. caliginosa	12.4	12.3	11.9
A. chlorotica	11.5	11.8	12.3
L. rubellus	-	15.4	-
YSTWYTH			
E. foetida	20.2	20.0	17.8
L. terrestris	21.8	20.0	22.4
A. longa	26.8	27.4	16.6
A. chlorotica	15.9	14.3	11.6
HAILKYN MT.			
E. foetida	62.2	61.3	66.4
L. terrestris	73.7	72.1	65.2
A. longa	74.9	64.1	29.8
SHIPHAM			
E. foetida	51.6	40.1	50.8
L. terrestris	101	88.0	86.1
A. longa	79.4	44.9	27.0
A. caliginosa	53.7	25.4	26.5
PARYS MT.			
E. foetida	210	218	207
L. terrestris	218	238	170
A. longa	237	180	79.9
B-POLDER			
E. foetida	64.7	57.3	56.2
L. terrestris	68.8	42.4	37.6
A. longa	54.0	57.6	-
L. rubellus	-	52.4	-
A-POLDER			
E. foetida	38.8	78.3	26.6
L. terrestris	-	40.0	-
A. longa	48.0	41.1	20.4
A. caliginosa	-	25.3	-

ELEMENT - COPPER (Continued)

	DAY 15	DAY 28	DAY 56
S-POLDER			
E. foetida	33.0	26.4	29.0
L. terrestris	43.1	37.2	35.8
A. longa	-	34.4	-
A. caliginosa	37.0	16.6	18.6
N-SLUDGE			
E. foetida	94.4	73.8	83.5
L. terrestris	-	97.0	-
A. longa	93.1	83.2	65.7
A. chlorotica	41.9	28.5	41.8

ELEMENT - NICKEL

	DAY 15	DAY 28	DAY 56
FRONGOCH			
E. foetida	9.21	11.9	9.93
L. terrestris	11.4	11.9	11.6
A. longa	12.2	9.04	7.68
A. caliginosa	10.5	10.3	11.9
A. chlorotica	8.7	9.14	11.7
L. rubellus	-	15.8	-
YSTWYTH			
E. foetida	15.3	14.4	11.8
L. terrestris	20.0	17.8	16.5
A. longa	23.0	24.7	11.9
A. chlorotica	13.1	10.5	6.93
HALKYN MT.			
E. foetida	21.8	19.3	18.6
L. terrestris	31.1	29.9	25.4
A. longa	30.0	23.5	2.17
SHIPHAM			
E. foetida	5.53	4.99	10.7
L. terrestris	11.9	11.3	9.34
A. longa	9.31	4.87	2.41
A. caliginosa	7.48	2.61	4.83
PARYS MT.			
E. foetida	4.18	4.59	8.18
L. terrestris	6.75	6.52	4.02
A. longa	5.42	4.38	2.34
B-POLDER			
E. foetida	10.9	9.08	10.7
L. terrestris	13.7	6.50	3.86
A. longa	8.98	10.0	-
L. rubellus	-	13.6	-
A-POLDER			
E. foetida	8.65	7.38	11.7
L. terrestris	-	12.1	-
A. longa	14.5	11.7	2.38
A. caliginosa	-	6.14	-

ELEMENT - NICKEL (Continued)

	DAY 15	DAY 28	DAY 56
S-POLDER			
E. foetida	6.52	1.40	6.67
L. terrestris	13.1	11.5	10.0
A. longa	-	9.32	-
A. caliginosa	6.61	3.40	5.03
N-SLUDGE			
E. foetida	16.5	10.7	14.4
L. terrestris	-	20.6	-
A. longa	18.1	15.3	8.59
A. chlorotica	7.26	3.65	10.9

ELEMENT - CADMIUM

	DAY 15	DAY 28	DAY 56
FRONGOCH			
E. foetida	<0.13	0.19	0.99
L. terrestris	3.24	4.24	2.28
A. longa	2.48	8.48	5.53
A. caliginosa	4.82	6.55	6.20
A. chlorotica	4.85	6.18	6.31
L. lrubellus	-	1.70	-
YSTWYTH			
E. foetida	<0.13	0.91	1.80
L. terrestris	3.49	4.61	6.62
A. longa	3.55	3.62	15.7
A. chlorotica	5.77	10.2	17.9
HALKYN MT.			
E. foetida	3.37	5.41	7.25
L. terrestris	5.86	9.18	12.5
A. longa	8.84	10.4	16.6
SHIPHAM			
E. foetida	383	276	339
L. terrestris	890	843	842
A. longa	671	398	275
A. caliginosa	525	259	258
PARYS MT.			
E. foetida	<0.13	<0.13	<0.13
L. terrestris	0.48	1.16	6.79
A. longa	1.77	2.93	10.8
B-POLDER			
E. foetida	6.13	7.48	11.6
L. terrestris	12.2	9.31	13.4
A. longa	11.6	15.7	-
L. rubellus	-	8.52	-
A-POLDER			
E. foetida	5.01	6.03	12.3
L. terrestris	-	10.4	-
A. longa	11.4	11.2	22.6
A. caliginosa	-	16.0	-

ELEMENT - CADMIUM (Continued)

	DAY 15	DAY 28	DAY 56
S-POLDER			
E. foetida	2.88	3.40	4.90
L. terrestris	6.62	6.37	5.59
A. longa	-	10.8	-
A. caliginosa	13.4	16.3	15.7
N-SLUDGE			
E. foetida	14.8	16.4	22.2
L. terrestris	-	20.2	-
A. longa	20.2	22.8	23.5
A. chlorotica	12.4	14.0	19.6

ELEMENT - CHROMIUM

	DAY 15	DAY 28	DAY 56
FRONGOCH			
E. foetida	19.9	17.8	70.4
L. terrestris	20.1	22.4	25.9
A. longa	20.5	17.2	16.5
A. caliginosa	-	19.0	20.4
A. chlorotica	15.6	16.8	19.7
L. rubellus	-	22.3	-
YSTWYTH			
E. foetida	17.5	15.9	16.9
L. terrestris	22.4	22.3	24.3
A. longa	24.2	31.9	16.0
A. chlorotica	17.1	12.9	7.77
HALKYN MT.			
E. foetida	19.3	14.4	19.5
L. terrestris	23.6	25.5	23.8
A. longa	19.3	21.2	3.85
SHIPHAM			
E. foetida	9.17	9.65	12.3
L. terrestris	17.3	14.9	14.1
A. longa	13.7	9.18	4.58
A. caliginosa	-	5.24	5.26
PARYS MT.			
E. foetida	15.9	16.3	17.3
L. terrestris	18.3	19.6	15.6
A. longa	15.4	14.2	14.2
B-POLDER			
E. foetida	52.4	61.2	52.1
L. terrestris	97.4	52.7	35.0
A. longa	63.6	71.3	-
L. rubellus	-	69.8	-
A-POLDER			
E. foetida	76.3	42.1	24.3
L. terrestris	-	70.4	-
A. longa	73.2	65.7	12.5
A. caliginosa	-	35.5	-

ELEMENT - CHROMIUM (Continued)

	DAY 15	DAY 28	DAY 56
S-POLDER			
E. foetida	21.1	8.8	14.4
L. terrestris	39.6	35.7	33.0
A. longa	-	27.6	-
A. caliginosa	-	13.5	12.1
N-SLUDGE			
E. foetida	121	75.2	82.3
L. terrestris	-	161	-
A. longa	123	101	58.0
A. chlorotica	50.5	22.4	31.0

ELEMENT - LEAD

	DAY 15	DAY 28	DAY 56
FRONGOCH			
E. foetida	29.6	27.6	28.0
L. terrestris	34.4	36.6	35.9
A. longa	40.5	35.1	29.9
A. caliginosa	39.0	33.2	35.7
A. chlorotica	29.0	30.1	35.8
L. rubellus	-	49.0	-
YSTWYTH			
E. foetida	651	696	829
L. terrestris	760	702	779
A. longa	934	935	696
A. chlorotica	548	567	599
HALKYN MT.			
E. foetida	359	312	291
L. terrestris	494	492	438
A. longa	543	398	61.7
SHIPHAM			
E. foetida	13141	9475	12025
L. terrestris	15421	14849	21158
A. longa	15965	11188	5564
A. caliginosa	15122	5865	4887
PARYS MT.			
E. foetida	582	599	567
L. terrestris	597	659	436
A. longa	687	488	223
B-POLDER			
E. foetida	55.8	43.6	36.4
L. terrestris	82.2	39.5	29.1
A. longa	56.3	65.4	-
L. rubellus	-	63.4	-
A-POLDER			
E. foetida	34.7	24.8	12.1
L. terrestris	-	47.2	-
A. longa	68.1	48.3	46.3
A. caliginosa	-	32.5	-

ELEMENT - LEAD (Continued)

	DAY 15	DAY 28	DAY 56
S-POLDER			
E. foetida	36.7	9.52	21.4
L. terrestris	80.8	69.5	63.6
A. longa	-	62.2	-
A. caliginosa	48.7	25.8	25.6
N-SLUDGE			
E. foetida	44.4	26.4	30.4
L. terrestris	-	64.0	-
A. longa	59.4	46.9	30.9
A. chlorotica	138	7.57	10.6

APPENDIX II

THE USE OF ACID INSOLUBLE RESIDUE TO CORRECT FOR THE PRESENCE OF SOIL-DERIVED METALS IN THE GUT OF EARTHWORMS USED AS BIO-INDICATOR ORGANISMS IN STUDIES OF HEAVY METAL MOBILITY IN THE SOIL

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ABSTRACT

Biological availability of heavy metals to earthworms can only be determined after complete separation of earthworm tissue from soil within the earthworm gut. In previous studies, this has been done most commonly by either starvation, to allow for depuration of the earthworm gut or dissection to remove soil from within the gut. The use of acid insoluble residue (A.I.R.) as a marker fraction in the soil is proposed for correcting measurements of the heavy metals: Zn, Cu, Cd and Pb in earthworm samples to eliminate the contribution from soil in the earthworm gut. Five species of earthworm were kept for 15 days on four experimental soils which contained different levels of Zn, Cu, Cd and Pb. Results of heavy metal concentrations in earthworm tissue obtained after calculation using the correction based on A.I.R. content were compared statistically with results obtained by other methods. In the majority of soil/earthworm combinations no significant difference emerged between the calculated results and those obtained after dissection of earthworm samples, suggesting that the correction using A.I.R. content provides a suitable method for estimating levels of heavy metals present in earthworm tissue.

INTRODUCTION

The initial reason for studying metal concentrations in earthworm samples dictates whether or not it is necessary to separate earthworm tissue from soil within the earthworm gut and the method used to achieve this separation prior to heavy metal analysis. Studies evaluating levels of contaminants in biota at varying distances from a pollutant source e.g. a busy road (Gish & Christensen, 1973; Ash & Lee, 1980), an industrial emission source (Bull et al., 1977; Wright & Stringer (1980) or mine wastes (Ireland & Wooton, 1976; Roberts & Johnson, 1978) include measurements of heavy metals in whole worm samples including some soil within the gut. In food chain studies the metal concentrations of interest to the investigator would be the earthworm, including gut contents, as ingested by a predator at the next trophic level. In this case no separation of earthworm tissue from soil is necessary (Martin & Coughtry, 1976; Roberts & Johnson, 1978).

Recently, however, earthworms have been used in a number of studies to indicate biologically available levels of contaminants either applied to or present in soils and sediments (Czarnowska and Jopkiewicz, 1978; Atlanvinyte et al, 1980; Carter et al., 1980, Marquenie and Simmers, 1984; Pietz et al., 1984). As bioassay and biomonitoring organisms earthworms possess many characteristics which make them highly suitable for assessing the bioavailability of contaminants present within soils and sediments (Ma, 1982). However, after removal of the earthworms from the substrate and prior to chemical analysis it is necessary to separate the earthworm tissue from soil present within the gut. Unless adequate separation is achieved, results will reflect the amounts of contaminants present in the soil within the gut (not necessarily bio-available) as well as contaminants present within the earthworm tissue (bio-available). If a contaminant is not bioavailable, but present in very large concentrations in a soil, its apparent bioavailability will be greatly overestimated if even small amounts of soil remain in the gut.

Several methods are currently used to separate soil from tissue, the most common being dissection (Tarradellas et al., 1982) and starvation to allow for gut evacuation (Yadav et al., 1976; Hartenstein et al., 1980). Both methods are slow and laborious and the accuracy of results obtained after starving the earthworms is investigated in the present study. Marker elements, which are present in the soil in relatively high concentrations, but are biologically unavailable to the earthworm have been suggested as an alternative method for correcting for soil within the gut (Helmke et al., 1979; Beyer et al., 1982).

In the present investigation acid insoluble residue (A.I.R.) was used as a 'marker' to calculate the concentration of Zn, Cu, Cd and Pb in earthworm samples which could be attributed to soil within the gut. Consequently the concentration of these metals in the earthworm tissue (bio-available) may also be determined. The use of acid insoluble residue as a marker fraction for correcting 'whole worm' analyses for the presence of soil was compared with results obtained by analyses of dissected earthworms from which the soil was removed.

MATERIALS AND METHODS

SOILS One soil with relatively low levels of heavy metals (Frongoch: Aberystwyth, Dyfed, Wales) and three soils with high concentrations of one or more of the elements Zn, Cu, Cd and Pb (Ystwyth: Ystwyth Valley, Dyfed, Wales); Shipham (Shipham, Somerset, England) and Broek Polder (Rotterdam, Holland) were chosen. All soils were air dried and passed through a 2 mm mesh sieve; their chemical and physical characteristics are given in Table 1.

EARTHWORMS Five species of earthworm were selected for use in the experiment: Lumbricus terrestris (Linnaeus, 1758); Allolobophora longa (Ude, 1885); Allolobophora caliginosa (Savigny, 1826) and Allolobophora chlorotica (Savigny, 1826). These earthworms were collected by application of a 0.5% formaldehyde solution to the soil in Rothamsted Park. This soil contained 120 ug g^{-1} Zn; 26.9 ug g^{-1} Cu; <0.13 ug g^{-1} Cd and 52 ug g^{-1} Pb, i.e. values which were closely similar to those in the Frongoch soil. Earthworms emerging as a result of the vermifuge were immediately placed in tap water before being separated according to species. Eisenia foetida (Savigny, 1826) was collected from cattle manure. All earthworms were rinsed several times in distilled water and mature, clitellate individuals were selected for experimental use.

EXPERIMENTAL PROCEDURE Groups of each species of earthworm were placed on separate sub-samples of each soil and those which had not burrowed into the soil within five minutes were replaced. A ratio of approximately 5 gms (live weight) of earthworms to 600 gms of soil was used because (air dry weight) it was estimated from previous studies (Curry and Bolger, 1984 E.E.C. Directive, 1984; Marquenie and Simmers, 1984; Neuhauser et al., 1984) to be in excess of the earthworms' feeding requirements during the fifteen day experimental period. Three to five replicates of the following combinations of earthworms and experimental soils were used:- L. terrestris in each of the four soils; A. longa and A. caliginosa in the Frongoch soil; A. chlorotica in the Ystwyth soil and E. foetida in the Shipham soil. An experimental period of fifteen

days (at a constant temperature of 15°C) was considered sufficient to ensure that earthworms had egested all traces of the original soil from which they were collected and fully ingested experimental soil.

After 15 days earthworms were hand sorted from each soil and the worms from each replicate divided into three groups which were treated as follows:-

'whole worm' - Immediately after removal from the soil earthworms were rinsed in distilled water, killed and oven dried at 85°C to constant weight. These 'whole worm' samples comprised both earthworm tissue and soil within the alimentary canal.

'starved worm' - Earthworms were placed in clean petri dishes at 100% humidity (a few ml of distilled water) for 48 hours (transferred to clean petri dishes after the first 24 hours). All soil egested by the earthworms during the 48 hour period was collected, dried and analysed for acid insoluble residue. Earthworms which had been starved for 48 hours were rinsed in distilled water, killed and oven dried at 85°C to constant weight.

'dissected worm' - Earthworms were dissected along the entire length of the alimentary canal which was rinsed free of soil with distilled water before being oven dried at 85°C to constant weight.

CHEMICAL ANALYSIS

Acid Insoluble Residue (A.I.R.): Oven dried soil samples (ingested and egested soil) and whole, starved and dissected worm samples (approx. 0.5g) were ashed at 450°C overnight, the ash digested in 5 ml 6N HCl at 100°C for 1 hour and taken to dryness. Samples were re-extracted in hot 5% HCl, filtered and washed free of acid through Whatman No. 42 paper. Residue and filter paper were re-ignited at 600°C, A.I.R. determined gravimetrically and expressed as a percentage of the oven-dry weight. Acid and filter paper blanks were also run.

Heavy metal determination: All oven dried soil and earthworm samples (approx. 0.5 g) were wet ashed with 'AnalaR' grade concentrated HNO_3 (8 ml) for 5 hours at 125°C. After cooling, 'AnalaR' grade 70% $HClO_4$ (1 ml) was added before reheating to 200°C and taking samples almost to dryness. Samples were re-extracted with hot 5% HCl and concentrations of Al, Fe, Zn, Cu, Cd and Pb determined by inductively coupled plasma (ICP) optical emission spectrometry (ARL 34000 instrument). Reagent blanks were also analysed.

STATISTICAL ANALYSIS - Significant differences ($p < 0.05$) between the means given in Table 5 were calculated using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS

Potential of Acid Insoluble Residue as a 'marker' fraction

The acid insoluble residue fraction varied from 25% to 77% of the oven dry weight between soils but there was little variation within each soil (Table 2). Analysis of soils egested by the various species of earthworm indicated that the percentage of A.I.R. remained unchanged as a result of the soil having been ingested by the earthworms (Table 2) hence chemical analyses of soils prior to ingestion were considered sufficiently accurate for use in subsequent calculations.

Acid insoluble residue measured in dissected earthworms ranged from 0.80% to 1.15% with a mean value for all species of 1% (Table 2). In order to obtain an independent estimate of the acid insoluble residue content of soil-free earthworm tissue, linear regressions were calculated for acid insoluble residue content of earthworm samples containing increasing quantities of soil (dissected, starved and whole worm samples) on Al concentrations of these samples. A strong linear relationship emerged (Table 3) and the y- intercept values (A.I.R. content of Al, (and therefore soil-free earthworm) were small, ranging from 0.29% $\pm$ 0.23% to 0.84% $\pm$ 0.70%.

The results used in the linear regression analysis included data for Al and A.I.R. content of earthworms starved for 48 hours to allow for gut depuration. While this may be a sufficient period for eliminating soil from the gut of smaller species of earthworm e.g. *E. foetida*, relatively high concentrations of A.I.R., Fe and Al suggest that some soil was still present within the gut of the larger field species of earthworm such as *L. terrestris* (Table 4).

Calculation to correct for metal concentration in the sample resulting from soil within the earthworm gut

Knowing the acid insoluble residue content of a soil and of an earthworm sample taken from that soil and having established that the acid insoluble residue content of a soil-free earthworm is approx. 1% by weight (and therefore considered negligible), the absolute quantity of soil in the whole worm sample may be calculated as follows:-

$$\text{Weight of soil in sample (g)} = \text{Weight of sample (g)} \times \frac{\text{A.I.R. in sample (\%)}}{\text{A.I.R. in soil (\%)}}$$

This weight may then be used, together with the known concentration of any element (X) in the soil, to calculate the absolute quantity of element X in the earthworm sample which may be attributed to soil. The concentration of element X in soil-free earthworm tissue may then be calculated as follows:-

$$\text{Conc. of element X in worm tissue } (\mu g\ g^{-1}) = \frac{(\text{Wt of total sample (g)} \times \text{Conc. of element X in total sample } (\mu g\ g^{-1})) - (\text{Wt of soil in sample (g)} \times \text{Conc. of element X in soil } (\mu g\ g^{-1}))}{(\text{Wt of total sample (g)} - \text{Wt of soil in sample (g)})}$$

The above calculation was applied to each of the whole worm samples for the elements Zn, Cu, Cd and Pb. Results thus obtained are given in the "calculated treatment" in Table 5 (mean value of 3 to 5 replicates). These results have been statistically compared with metal concentrations measured in the whole, starved and dissected earthworm samples obtained at the end of the 15-day experimental period (Table 5).

With the exception of earthworms held in the Shipham soil which contained extremely high concentrations of Zn, little significant difference ($p < 0.05$) emerged between the Zn concentrations measured in whole, starved and dissected earthworms and the Zn concentration of soil-free earthworm tissue obtained by calculation. A similar pattern was evident for the concentrations of Cd measured in the earthworm samples (Table 5). However, Cu and Pb concentrations were observed to increase in earthworm samples as the amount of soil in the sample increased (Table 5). A general pattern emerged in which 'whole worm' samples contained significantly higher ($p < 0.05$) concentrations of Cu and Pb than 'starved worm' samples and both these values were significantly higher ($p < 0.05$) than Pb and Cu concentrations measured in dissected earthworms and values for soil-free earthworms obtained by calculation (Table 5).

In the majority of combinations of earthworm species and soil, the concentration of Zn, Cu, Cd and Pb measured in the dissected earthworm was not significantly different ($p < 0.05$) from the value obtained using the above calculation (Table 5). Only in the following combinations: Cu/*L. terrestris*/Frongoch; Pb/*L. terrestris*/Shipham; Pb/*E. foetida*/Shipham and Zn/*E. foetida*/Shipham were the two values significantly different ($p < 0.05$).

DISCUSSION

Elements such as Ti, Al and Fe, present at relatively high levels in soil compared with plant and animal tissues, or fractions of the soil, such as ash and A.I.R. have been used in studies measuring metal concentrations in plant tissue samples and animal forages to provide some indication of the extent to which soil is present in the sample (McGrath *et al.*, 1982; Cherney & Robinson, 1983; Cherney *et al.*, 1983). In a similar context, both ash (Beyer *et al.*, 1982) and the element Sc (Helmke *et al.*, 1979) have been suggested for use in studies of heavy metal uptake into earthworm tissues. Artificial markers such as Cr_2O_3 and various dyes have been added to earthworm substrate to measure the rate of passage of substrate through the earthworm gut (Hartenstein *et al.*, 1981; Hartenstein & Amico, 1983) but these may act as irritants resulting in accelerated egestion rates (M. Morgan, pers. comm. 1985). A marker which occurs naturally in the substrate of the earthworm was selected in this study to minimize such effects.

A preliminary investigation was conducted to evaluate the possibility of using either Ti, Al, ash or A.I.R. concentration as a method of correcting for soil within earthworm samples but concentrations of the first three

measured in dissected, soil-free earthworm tissue were high relative to the respective concentrations in the soils, and their use would introduce an unacceptable degree of error into the final calculation.

The mean A.I.R. content of dissected, soil-free earthworm was 1% and was assumed to be negligible for the purposes of calculating the quantity of soil present in a 'whole worm' sample. The error introduced into the calculation by making this assumption will increase in magnitude as the A.I.R. content of the soil decreases. If, in addition, the element X for which the calculation is made is present in soil at very high concentrations, as is the case for Zn and Pb in the Shipham soil (142,637 ug g^{-1} and 22,531 ug g^{-1} respectively), then the error will increase proportionately and may even result in negative values, e.g. the Zn/E. foetida/Shipham combination (Table 5). Therefore, where a combination of relatively low A.I.R. and very high metal concentration in the soil occurs, the magnitude of error introduced into the calculation will limit the accuracy of the result.

For the purposes of this investigation the heavy metal concentration measured in a dissected earthworm, washed free of soil from within the gut, was considered the most accurate method of directly measuring the heavy metal concentration in soil-free earthworm tissue. In most cases, when these results were statistically compared with results obtained by calculation, using concentrations of heavy metals measured in 'whole worm' samples, no significant difference emerged (Table 5). This suggests that A.I.R. can be used to correct for metals present in an earthworm sample as a result of soil in the sample. Metal concentrations measured in whole worm samples (Table 5) clearly demonstrate the necessity for separating earthworm tissue from soil present within the earthworm gut when the bio-availability of heavy metals is being determined.

The majority of studies measuring heavy metal concentrations in earthworm tissues have used either dissection and washing of earthworms at the end of the experiment or storing earthworms on clean/inert substrates prior to heavy metal analysis (Table 6). Both are time consuming and have several other disadvantages. In the dissection method the folds of the typhlosole within the earthworms' alimentary canal make total removal of soil from the gut difficult and excessive handling may result in loss of the chlorogogenous tissue associated with the gut wall which is involved in the storage of heavy metals, in particular Pb (Ireland, 1975; Ireland & Richards, 1977). Results in Table 5 suggest that this may have occurred in the present experiment since Pb concentrations measured in the dissected earthworm samples were consistently, but not significantly, lower than Pb concentrations obtained by calculation.

Starving the earthworm to allow for gut evacuation is the method which has been most commonly used (Table 6) but results in Tables 4 and 5 provide some evidence that after 48 hours soil still remains within the gut of L. terrestris, making a significant contribution to the heavy metal content of the sample. Longer periods of starvation used by some authors (Table 6) may result in depuration of heavy metals from the earthworm tissue (Richards and Ireland, 1978). Results from the present experiment suggest that, at least for the larger species of earthworm L. terrestris a more accurate estimation of metal concentration in earthworm tissue is obtained either by calculation or by dissection compared with starving the earthworms for 48 hours prior to analysis (Table 5).

Although some uptake of heavy metals from the experimental soils into earthworm tissue will have occurred during the experimental period fifteen days is likely to be insufficient time for any equilibrium to have been reached between soil and earthworm metal concentrations (Marquenie & Simmers, 1984). Therefore no detailed discussion is made here of comparative uptake of heavy metals into earthworm tissue between the various combinations of earthworm species and experimental soils.

High base line levels of Zn and Cd were recorded in earthworms used in this study despite their having been collected from an unpolluted soil. Some bioaccumulation is likely to have occurred during the life span of these worms. Evidence for accumulation of Zn and Cd from unpolluted soils have been observed by other investigators (Helmke et al., 1979; Carter et al., 1980; Ma, 1982; Pietz et al., 1984).

Zinc and cadmium concentrations measured in earthworms held in Frongoch, Ystwyth and Brock-Polder soils showed no signficant difference ($p < 0.05$) between 'whole worm', 'starved worm', 'dissected worm' and calculated values (Table 5). In the Frongoch soil the Zn and Cd concentrations were smaller than that of the earthworm tissue and under these circumstances soil within the gut effectively diluted and reduced the metal concentration measured in 'whole worm' samples compared with 'dissected worm' and calculated values. In the Ystwyth and Brock Polder soils similar levels of Zn and Cd were present in soil and earthworm tissue and soil within the worm gut exerted little influence on the metal concentration measured in the variously treated worm samples. However, when concentrations of Zn and Cd in the soil were much greater than those in the tissue (earthworms in Shipham soil) soil within the gut significantly elevated the metal concentration of 'whole worm' samples compared with 'dissected worm' and calculated values.

Copper and lead concentrations in earthworm tissue were consistently smaller than those measured in the soils (Tables 2 and 5). These elements may be regulated at low levels by the earthworms or be biologically unavailable for uptake. In every soil/earthworm combination soil within the worm gut resulted in increased metal concentrations in 'whole worm' samples compared with 'starved worm', 'dissected worm' and calculated values.

CONCLUSION

The method outlined in this paper, using metal concentrations in whole worm samples and soils, and A.I.R. in the sample, gives results not significantly different from values measured in dissected, soil-free earthworms. In addition, using this method will provide information both on the metal concentration of earthworms including gut contents, of use in food chain studies, as well as (after calculation) metal concentration of soil-free earthworm tissue.

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Table 1 Physical and chemical characteristics of the experimental soils

		FRONGOCH	YSTWYTH	BROEK-POLDER	SHIPHAM
Air dry soil < 2 mm					
Moisture (105°C) (%)		8.77	7.06	19.84	8.84
Particle size (%)	500µm-2µm	9	6	<1	26
	200µm-500µm	5	30	2	24
	100µm-200µm	2	<1	<1	14
	50µm-100µm	4	<1	9	8
	10µm-50µm	18	23	22	16
	2µm-16µm	38	32	35	11
	<2µm	24	9	32	1
Organic carbon (%C)		4.3	2.0	5.7	0.7
pH (1 : 2.5) in water		6.0	6.2	7.4	7.6
pH (1 : 2.5) in 0.01M $CaCl_2$		5.5	5.4	7.3	7.2
Cation exchange capacity (m.e./100g)		20.8	7.3	29.2	7.7
Oven (105°C) dry soil < 2 mm					
Metal concentrations (µg g^{-1}, dry weight)					
	Zn	116.0	790.2	828.8	142674
	Cu	22.9	34.7	153.8	207
	Cd	0.9	3.0	10.5	1617
	Pb	75.9	1442.1	222.3	22531

Table 2 Comparison of the acid insoluble residue (A.I.R.) (%) in ingesta and egesta of earthworms from each soil and A.I.R. (%) of earthworm tissue dissected and washed free of soil within the gut.

Soil	Earthworm species	A.I.R. of ingesta (soil)	A.I.R. of egesta ($\bar{x}$ ± standard deviation)	A.I.R. of dissected soil-free earthworm ($\bar{x}$ ± s.d.)
FRONGOCH	*L. terrestris*	73.6 ± 0.16	74.5 ± 1.39	0.9 ± 0.09
	A. longa		72.8 ± 0.89	0.9 ± 0.15
	A. caliginosa		71.1 ± 2.52	1.1 ± 0.06
YSTWYTH	*L. terrestris*	76.7 ± 0.19	78.3 ± 1.74	1.0 ± 0.23
	A. chlorotica		74.2 ± 1.92	1.2 ± 0.19
BROEK POLDER	*L. terrestris*	60.5 ± 0.23	57.8 ± 3.27	0.8 ± 0.34
SHIPHAM	*L. terrestris*	25.4 ± 0.21	24.4 ± 1.27	1.0 ± 0.34
	E. foetida		23.6 ± 2.18	1.1 ± 0.19

All results expressed as % over (105°C) dry weight

Table 3 Correlation co-efficients and Y-intercepts from linear regression analysis of acid insoluble residue against Al concentrations of whole, starved and dissected earthworms from each soil.

Soil	Correlation Co-efficient	Y-intercept (± s.d.)
FRONGOCH	0.998	0.39 ± 0.26
YSTWYTH	0.999	0.29 ± 0.23
BROEK POLDER	0.958	0.84 ± 0.70
SHIPHAM	0.971	0.28 ± 0.37

Table 4 Metal concentrations (μg g^{-1}, dry weight) and acid insoluble residue (A.I.R., % dry weight) of whole, starved and dissected L. terrestris after 15 days in each soil

Soil	Earthworm species	Treatment	A.I.R. (%)	Al	Fe
FRONGOCH	L. terrestris	Whole	30.2	10,587	17,407
		Starved	7.0	2,435	4,127
		Dissected	0.9	188	575
YSWYTH	L. terrestris	Whole	41.3	12,758	27,111
		Starved	8.6	2,673	5,393
		Dissected	1.0	143	609
BROEK-POLDER	L. terrestris	Whole	10.2	2,859	4,736
		Starved	4.7	1,284	2,186
		Dissected	0.8	57	306
SHIPHAM	L. terrestris	Whole	10.2	933	50,263
		Starved	5.8	670	31,732
		Dissected	1.0	45	1,311

Table 5 Metal concentrations (μg g^{-1}, dry weight) of whole, starved and dissected earthworms held in the four soils for 15 days compared with metal concentrations (μg g^{-1}, dry weight) calculated* to be present only in the earthworm tissue of the whole worm samples

Soil	Earthworm species	Treatment	Zn	Cu	Cd	Pb
FRONGOCH	L. terrestris	Whole	394.8 ^{a}	12.8 ^{d}	6.4 ^{a}	42.0 ^{c}
		Starved	506.9 ^{a}	9.1 ^{c}	9.1 ^{a}	<6.6 ^{a}
		Dissected	636.8 ^{a}	7.4 ^{b}	8.5 ^{a}	12.6 ^{ab}
		Calculated	600.9	5.7 ^{a}	10.4 ^{a}	18.3 ^{b}
			(89.08)	(0.44)	(1.67)	(2.99)
	A. longa	Whole	565.4 ^{a}	13.4 ^{b}	10.3 ^{a}	42.9 ^{b}
		Dissected	723.0 ^{a}	8.1 ^{a}	12.1 ^{a}	10.9 ^{a}
		Calculated	834.0 ^{a}	7.7	15.8	22.9
			(105.06)	(1.17)	(2.65)	(3.14)
	A. caliginosa	Whole	429.0 ^{a}	13.2 ^{b}	9.0 ^{a}	34.5 ^{b}
		Dissected	503.0 ^{a}	8.7 ^{a}	11.4 ^{a}	<13.2 ^{a}
		Calculated	568.5	8.7 ^{a}	12.7 ^{a}	16.1 ^{a}
			(57.00)	(0.53)	(1.75)	(2.68)
YSTWYTH	L. terrestris	Whole	763.5 ^{a}	22.2 ^{c}	6.6 ^{a}	798.0 ^{c}
		Starved	716.3 ^{a}	12.6 ^{b}	9.5 ^{b}	196.6 ^{b}
		Dissected	828.5 ^{a}	7.6 ^{a}	11.6 ^{b}	76.7 ^{a}
		Calculated	720.5 ^{a}	7.7 ^{a}	10.9	147.2 ^{ab}
			(104.86)	(0.60)	(0.84)	(37.80)
	A. chlorotica	Whole	424.8 ^{b}	11.3 ^{b}	9.8 ^{a}	330.0 ^{b}
		Dissected	345.2 ^{ab}	9.1 ^{a}	9.9 ^{a}	162.6 ^{a}
		Calculated	373.1	8.1 ^{a}	10.6 ^{a}	185.0 ^{a}
			(20.78)	(0.29)	(0.84)	(16.89)
BROEK-POLDER	L. terrestris	Whole	675.9 ^{a}	38.6 ^{b}	12.1 ^{a}	44.3 ^{b}
		Starved	496.6 ^{a}	23.5 ^{ab}	9.3 ^{a}	16.8 ^{a}
		Dissected	667.7 ^{a}	10.7 ^{a}	9.3 ^{a}	<6.6 ^{a}
		Calculated	643.2 ^{a}	15.4	12.4 ^{a}	8.5
			(71.59)	(4.48)	(1.43)	(7.18)
SHIPHAM	L. terrestris	Whole	56,899.5 ^{c}	77.3 ^{c}	740.4 ^{c}	18,894.3 ^{b}
		Starved	31,953.0 ^{b}	48.1 ^{b}	434.4 ^{b}	12,618.7 ^{b}
		Dissected	2,642.0 ^{a}	13.9 ^{a}	60.2 ^{a}	779.7 ^{a}
		Calculated	4,663.8	7.8	157.8 ^{a}	17,219.5 ^{b}
			(635.53)	(6.85)	(80.99)	(2,063.29)
	E. foetida	Whole	24,036.8 ^{b}	49.6 ^{b}	310.0 ^{b}	10,755.2 ^{b}
		Dissected	1,658.6 ^{a}	14.1 ^{a}	32.6 ^{a}	872.5 ^{a}
		Calculated	neg.	19.2 ^{a}	133.0	8,052.0 ^{b}
			(1,947.29)	(2.65)	(35.40)	(799.36)

All figures are mean values (n = 3 to 5 replicates)

For each column values with the same superscript are not significantly different ($p<0.05$)

Standard Error given in parentheses

*For method of calculation see text

Table 6: Methods used to separate earthworms from soil within the earthworm gut in studies of heavy metal concentration in earthworm samples

Method	Authors
No gut depuration or method not stipulated	- Williamson & Evans (1972) - Martin & Coughtry (1976) - Roberts & Johnson (1978) - Andersen (1980) - Curry & Cotton (1980) - Wright & Stringer (1980)
Starved on a clean/inert substrate e.g. filter paper; distilled water	- Gish & Christensen (1973) refrigerated overnight - van Hook (1974) 4 days - Ireland (1975) 4 days at 17-19°C - Ireland & Hooton (1976) 4 days - van Rhee (1977) refrigerated overnight - Andersen (1979) - Ireland (1979) 4 days at 16-18°C - Ash & Lee (1980) 4 days at 20°C - Carter, Hayes & Laukulich (1980) 6 days at 15°C - Hartenstein, Neuhauser & Collier (1980) 4 days - Hartenstein et al (1980) overnight - Beyer, Chaney & Mulhern (1982) 1-3 days at 10°C - Fleckenstein & Graff (1984) Several days - Pietz et al (1984) 6-8 days at 23°C
Dissection to remove gut contents	- Czarnowska & Jopkiewicz (1978) - Andersen (1979) - Mori & Kurihara (1979)
Inter-element ratios to correct for soil present	- Helmke et al (1979) Sc - Beyer et al (1982) Ash

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